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$ISF_{deviation} = [(ISF_{actual})^2 + (ISF_{actual})^2]^{1/2}$.

Applying this equation to the values shown above, one can solve for the estimated 'true' value of the ISF error term:

$ISF_{actual} = [(ISF_{deviation})^2 - (Blood_{actual})^2]^{1/2}$.

Or, solving the equation,

$ISF_{actual} = [(13.4)^2 - (8.3)^2]^{1/2} = 10.5$ mg/dl.

A histogram of the relative deviation of the ISF to the blood glucose levels is shown in FIG. 27.

Drug Delivery through Pores in the Stratum Corneum

The present invention also includes a method for the delivery of drugs, including drugs currently delivered transdermally, through micro-pores in the stratum corneum. In one illustrative embodiment, the delivery is achieved by placing the solution in a reservoir over the poration site. In another illustrative embodiment, a pressure gradient is used to further enhance the delivery. In still another illustrative embodiment, sonic energy is used with or without a pressure gradient to further enhance the delivery. The sonic energy can be operated according to traditional transdermal parameters or by utilizing acoustic streaming effects, which will be described momentarily, to push the delivery solution through the porated stratum corneum.

Example 15

This example shows the use of stratum corneum poration for the delivery of lidocaine, a topical analgesic. The lidocaine solution also contained a chemical permeation enhancer formulation designed to enhance its passive diffusion across the stratum corneum. A drawing of an illustrative delivery apparatus 300 is shown in FIG. 28, wherein the apparatus comprises a housing 304 enclosing a reservoir 308 for holding a drug-containing solution 312. The top portion of the housing comprises an ultrasonic transducer 316 for providing sonic energy to aid in transporting the drug-containing solution through micropores 320 in the stratum corneum 324. A port 328 in the ultrasonic transducer permits application of pressure thereto for further aiding in transporting the drug-containing solution through the micropores in the stratum corneum. The delivery apparatus is applied to a selected area of an individual's skin such that it is positioned over at least one, and preferably a plurality, of micropores. An adhesive layer 332 attached to a lower portion of the housing permits the apparatus to adhere to the skin such that the drug-containing solution in the reservoir is in liquid communication with the micropores. Delivery of the drug through the micropores results in transport into the underlying epidermis 336 and dermis 340.

Five subjects were tested for the effectiveness of drug delivery using poration together with ultrasound. The experiment used two sites on the subjects left forearm about three inches

apart, equally spaced between the thumb and upper arm. The site near the thumb will be referred to as site 1 the site furthest from the thumb will be referred to as site 2. Site 1 was used as a control where the lidocaine and enhancer solution was applied using an identical delivery apparatus 300, but without any micro-poration of the stratum corneum or sonic energy. Site 2 was porated with 24 holes spaced 0.8 millimeters apart in a grid contained within a 1 cm diameter circle. The micropores in Site 2 were generated according to the procedure of Example 6. Lidocaine and low level ultrasound were applied. Ultrasound applications were made with a custom manufactured Zevex ultrasonic transducer assembly set in burst mode with 0.4 Volts peak to peak input with 1000 count bursts occurring at 10 Hz with a 65.4 kHz fundamental frequency, i.e., a pulse modulated signal with the transducer energized for 15 millisecond bursts, and then turned off for the next 85 milliseconds. The measured output of the amplifier to the transducer was 0.090 watts RMS.

After application of the lidocaine, sensation measurements were made by rubbing a 30 gauge wire across the test site. Experiments were executed on both sites, Site 1 for 10 to 12 minute duration and Site 2 for two 5 minute duration intervals applied serially to the same site. Both sites were assessed for numbness using a scale of 10 to 0, where 10 indicated no numbness and 0 indicated complete numbness as reported by the test subjects. The following summary of results is for all 5 subjects.

The control site, site 1, presented little to no numbness (scale 7 to 10) at 10 to 12 minutes. At approximately 20 minutes some numbness (scale 3) was observed at site 1 as the solution completely permeated the stratum corneum. Site 1 was cleaned at the completion of the lidocaine application. Site 2 presented nearly complete numbness (scale 0 to 1) in the 1 cm circle containing the porations. Outside the 1 cm diameter circle the numbness fell off almost linearly to 1 at a 2.5 cm diameter circle with no numbness outside the 2.5 cm diameter circle. Assessment of site 2 after the second application resulted in a slightly larger totally numb circle of about 1.2 cm diameter with numbness falling off linearly to 1 in an irregular oval pattern with a diameter of 2 to 2.5 cm perpendicular to the forearm and a diameter of 2 to 6 cm parallel to the forearm. Outside the area no numbness was noted. A graphic representation of illustrative results obtained on a typical subject is shown in FIGS. 29A-C. FIGS. 29A and 29B show the results obtained at Site 2 (porated) after 5 and 10 minutes, respectively. FIG. 29C shows the results obtained at Site 1 (control with no poration).

Sonic Energy and Enhancers for Enhancing Transdermal Flux

The physics of sonic energy fields created by sonic transducers can be utilized in a method by which sonic frequency can be modulated to improve on flux rates achieved by other methods. As shown in FIG. 1 of U.S. Patent No. 5,445,611, hereby incorporated herein by reference, the energy distribution of an sonic transducer can be divided into near and far fields.

The near field, characterized by length N, is the zone from the first energy minimum to the last energy maximum. The zone distal to the last maximum is the far field. The near (N) field pattern is dominated by a large number of closely spaced local pressure peaks and nulls. The length of the near field zone, N, is a function of the frequency, size, and shape of the transducer face, and the speed of sound in the medium through which the ultrasound travels. For a single transducer, intensity variations within its normal operating range do not affect the nature of the sonic energy distribution other than in a linear fashion. However, for a system with multiple transducers, all being modulated in both frequency and amplitude, the relative intensities of separate transducers do affect the energy distribution in the sonic medium, regardless of whether it is skin or another medium.

By changing the frequency of the sonic energy by a modest amount, for example in the range of about 1 to 20%, the pattern of peaks and nulls remains relatively constant, but the length N of the near field zone changes in direct proportion to the frequency. Major changes the frequency, say a factor of 2 or more, will most likely produce a different set of resonances or vibrational modes in the transducer, causing a significantly and unpredictably different near field energy pattern. Thus, with a modest change in the sonic frequency, the complex pattern of peaks and nulls is compressed or expanded in an accordion-like manner. By selecting the direction of frequency modulation, the direction of shift of these local pressure peaks can be controlled. By applying sonic energy at the surface of the skin, selective modulation of the sonic frequency controls movement of these local pressure peaks through the skin either toward the interior of the body or toward the surface of the body. A frequency modulation from high to low drives the pressure peaks into the body, whereas a frequency modulation from low to high pulls the pressure peaks from within the body toward the surface and through the skin to the outside of the body.

Assuming typical parameters for this application of, for example, a 1.27 cm diameter sonic transducer and a nominal operating frequency of 10 MHz and an acoustic impedance similar to that of water, a frequency modulation of 1 MHz produces a movement of about 2.5 mm of the peaks and nulls of the near field energy pattern in the vicinity of the stratum corneum. From the perspective of transdermal and/or transmucosal withdrawal of analytes, this degree of action provides access to the area well below the stratum corneum and even the epidermis, dermis, and other tissues beneath it. For any given transducer, there may be an optimal range of frequencies within which this frequency modulation is most effective.

The flux of a drug or analyte across the skin can also be increased by changing either the resistance (the diffusion coefficient) or the driving force (the gradient for diffusion). Flux can be enhanced by the use of so-called penetration or chemical enhancers.

Chemical enhancers are comprised of two primary categories of components, i.e., cell-envelope disordering compounds and solvents or binary systems containing both cell-envelope disordering compounds and solvents.

Cell envelope disordering compounds are known in the art as being useful in topical pharmaceutical preparations and function also in analyte withdrawal through the skin. These compounds are thought to assist in skin penetration by disordering the lipid structure of the stratum corneum cell-envelopes. A comprehensive list of these compounds is described in European Patent Application 43,738, published June 13, 1982, which is incorporated herein by reference. It is believed that any cell envelope disordering compound is useful for purposes of this invention.

Suitable solvents include water; diols, such as propylene glycol and glycerol; monoalcohols, such as ethanol, propanol, and higher alcohols; DMSO; dimethylformamide; N,N-dimethylacetamide; 2-pyrrolidone; N-(2-hydroxyethyl) pyrrolidone; N-methylpyrrolidone; 1-dodecylazacycloheptan-2-oneand other n-substituted-alkyl-azacycloalkyl-2-ones(azones) and the like.

U.S. Patent 4,537,776, Cooper, issued August 27, 1985, contains an excellent summary of prior art and background information detailing the use of certain binary systems for permeant enhancement. Because of the completeness of that disclosure, the information and terminology utilized therein are incorporated herein by reference.

Similarly, European Patent Application 43,738, referred to above, teaches using selected diols as solvents along with a broad category of cell-envelope disordering compounds for delivery of lipophilic pharmacologically-active compounds. Because of the detail in disclosing the cell-envelope disordering compounds and the diols, this disclosure of European Patent Application 43,738 is also incorporated herein by reference.

A binary system for enhancing metoclopramide penetration is disclosed in UK Patent Application GB 2,153,223 A, published August 21, 1985, and consists of a monovalent alcohol ester of a C8-32 aliphatic monocarboxylic acid (unsaturated and/or branched if C18-32) or a C6-24 aliphatic monoalcohol (unsaturated and/or branched if C14-24) and an N-cyclic compound such as 2-pyrrolidone, N-methylpyrrolidone and the like.

Combinations of enhancers consisting of diethylene glycol monoethyl or monomethyl ether with propylene glycol monolaurate and methyl laurate are disclosed in U.S. Patent 4,973,468 as enhancing the transdermal delivery of steroids such as progestogens and estrogens. A dual enhancer consisting of glycerol monolaurate and ethanol for the transdermal delivery of drugs is shown in U.S. Patent 4,820,720. U.S. Patent 5,006,342 lists numerous enhancers for transdermal drug administration consisting of fatty acid esters or fatty alcohol ethers of C_2 to C_4 alkanediols, where each fatty acid/alcohol portion of the ester/ether is of about 8 to 22 carbon atoms. U.S. Patent 4,863,970 shows penetration-enhancing compositions for topical application comprising an active permeant contained in a penetration-enhancing vehicle containing specified amounts of one or more cell-envelope disordering compounds such as oleic acid, oleyl alcohol, and glycerol esters of oleic acid; a C_2 or C_3 alkanol and an inert diluent such as water.

Other chemical enhancers, not necessarily associated with binary systems include DMSO or aqueous solutions of DMSO such as taught in Herschler, U.S. Patent 3,551,554; Herschler, U.S. Patent 3,711,602; and Herschler, U.S. Patent 3,711,606, and the azones (n-substituted-alkyl-azacycloalkyl-2-ones) such as noted in Cooper, U.S. Patent 4,557,943.

Some chemical enhancer systems may possess negative side effects such as toxicity and skin irritation. U.S. Patent 4,855,298 discloses compositions for reducing skin irritation caused by chemical enhancer containing compositions having skin irritation properties with an amount of glycerin sufficient to provide an anti-irritating effect.

Because the combination of microporation of the stratum corneum and the application of sonic energy accompanied by the use of chemical enhancers can result in an improved rate of analyte withdrawal or permeant delivery through the stratum corneum, the specific carrier vehicle and particularly the chemical enhancer utilized can be selected from a long list of prior art vehicles some of which are mentioned above and incorporated herein by reference. To specifically detail or enumerate that which is readily available in the art is not thought necessary. The invention is not drawn to the use of chemical enhancers per se and it is believed that all chemical enhancers, useful in the delivery of drugs through the skin, will function with dyes in optical microporation and also with sonic energy in effecting measurable withdrawal of analytes from beneath and through the skin surface or the delivery of permeants or drugs through the skin surface.

Example 16

Modulated sonic energy and chemical enhancers were tested for their ability to control transdermal flux on human cadaver skin samples. In these tests, the epidermal membrane had been separated from the human cadaver whole skin by the heat-separation method of Example 1. The epidermal membrane was cut and placed between two halves of the permeation cell with the stratum corneum facing either the upper (donor) compartment or lower (receiver) compartment. Modified Franz cells were used to hold the epidermis, as shown in FIG. 2 of U.S. Patent No. 5,445,611. Each Franz cell consists of an upper chamber and a lower chamber held together with one or more clamps. The lower chamber has a sampling port through which materials can be added or removed. A sample of stratum corneum is held between the upper and lower chambers when they are clamped together. The upper chamber of each Franz cell is modified to allow an ultrasound transducer to be positioned within 1 cm of the stratum corneum membrane. Methylene blue solution was used as an indicator molecule to assess the permeation of the stratum corneum. A visual record of the process and results of each experiment was obtained in a time stamped magnetic tape format with a video camera and video cassette recorder (not shown). Additionally, samples were withdrawn for measurement with an absorption spectrometer to quantitate the amount of dye which had traversed the stratum corneum membrane during an experiment.

Chemical enhancers suitable for use could vary over a wide range of solvents and/or cell envelope disordering compounds as noted above. The specific enhancer utilized was: ethanol/glycerol/water/glycerolmonooleate/methyl laurate in 50/30/15/2.5/2.5 volume ratios. The system for producing and controlling the sonic energy included a programmable 0-30 MHz arbitrary waveform generator (Stanford Reserach Systems Model DS345), a 20 watt 0-30 MHz amplifier, and two unfocused ultrasound immersion transducers having peak resonances at 15 and 25 MHz, respectively. Six cells were prepared simultaneously for testing of stratum corneum samples from the same donor. Once the stratum corneum samples were installed, they were allowed to hydrate with distilled water for at least 6 hours before any tests were done.

Example 17

Effects of Sonic Energy without Chemical Enhancers

As stated above in Example 16, the heat-separated epidermis was placed in the Franz cells with the epidermal side facing up, and the stratum corneum side facing down, unless noted otherwise. The lower chambers were filled with distilled water, whereas the upper chambers were filled with concentrated methylene blue solution in distilled water.

Heat Separated Epidermis: Immediately after filling the upper chambers with methylene blue solution, sonic energy was applied to one of the cells with the transducer fully immersed. This orientation would correspond, for example, to having the transducer on the opposite side of a fold of skin, or causing the sonic energy to be reflected off a reflector plate similarly positioned and being used to "push" analyte out of the other side of the fold into a collection device. The sonic energy setting was initially set at the nominal operating frequency of 25 MHz with an intensity equivalent to a 20 volt peak-to-peak (P-P) input wave form. This corresponds to roughly a 1 watt of average input power to the transducer and similarly, assuming the manufacturer's nominal value for conversion efficiency of 1% for this particular transducer, a sonic output power of around 0.01 watts over the 0.78 cm^2 surface of the active area or a sonic intensity of 0.13 $watts/cm^2$. Three other control cells had no sonic energy applied to them. After 5 minutes the sonic energy was turned off. No visual indication of dye flux across the stratum corneum was observed during this interval in any of the cells, indicating levels less than approximately 0.0015% (v/v) of dye solution in 2 ml of receiver medium.

Testing of these same 3 control cells and 1 experimental cell was continued as follows. The intensity of sonic energy was increased to the maximum possible output available from the driving equipment of a 70 volt peak-to-peak input 12 watts average power input or ($\approx$0.13 $watts/cm^2$) of sonic output intensity. Also, the frequency was set to modulate or sweep from 30 MHz to 10 MHz. This 20 MHz sweep was performed ten times per second, i.e., a sweep rate of 10 Hz. At these input power levels, it was necessary to monitor the sonic energy transducer to avoid overheating. A contact thermocouple was applied to the body of the transducer and power

was cycled on and off to maintain maximum temperature of the transducer under 42°C. After about 30 minutes of cycling maximum power at about a 50% duty cycle of 1 minute on and 1 minute off, there was still no visually detectable permeation of the stratum corneum by the methylene blue dye.

A cooling water jacket was then attached to the sonic energy transducer to permit extended excitation at the maximum energy level. Using the same 3 controls and 1 experimental cell, sonic energy was applied at maximum power for 12 hours to the experimental cell. During this time the temperature of the fluid in the upper chamber rose to only 35°C, only slightly above the approximately 31°C normal temperature of the stratum corneum in vivo. No visual evidence of dye flux through the stratum corneum was apparent in any of the four cells after 12 hrs. of sonic energy applied as described above.

Example 18

Effects of Sonic Energy without Chemical Enhancers

Perforated Stratum Corneum: Six cells were prepared as described above in Example 16. The clamps holding the upper and lower chambers of the Franz cells were tightened greater than the extent required to normally seal the upper compartment from the lower compartment, and to the extent to artificially introduce perforations and "pinholes" into the heat-separated epidermal samples. When dye solution was added to the upper chamber of each cell, there were immediate visual indications of leakage of dye into the lower chambers through the perforations formed in the stratum corneum. Upon application of sonic energy to cells in which the stratum corneum was so perforated with small "pinholes," a rapid increase in the transport of fluid through a pinhole in the stratum corneum was observed. The rate of transport of the indicator dye molecules was directly related to whether the sonic energy was applied or not. That is, application of the sonic energy caused an immediate (lag time approximately <0.1 second) pulse of the indicator molecules through the pinholes in the stratum corneum. This pulse of indicator molecules ceased immediately upon turning off of the sonic energy (a shutoff lag of approximately <0.1 second). The pulse could be repeated as described.

Example 19

Effects of Sonic Energy and Chemical Enhancers

Two different chemical enhancer formulations were used. Chemical Enhancer One or CE1 was an admixture of ethanol/glycerol/water/glycerol monooleate/methyl laurate in a 50/30/15/2.5/2.5 volume ratio. These are components generally regarded as safe, i.e. GRAS, by the FDA for use as pharmaceutical excipients. Chemical Enhancer Two or CE2 is an experimental formulation shown to be very effective in enhancing transdermal drug delivery, but generally considered too irritating for long term transdermal delivery applications. CE2 contained

ethanol/glycerol/water/lauradone/methyllaurate in the volume ratios 50/30/15/2.5/2.5. Lauradone is the lauryl (dodecyl) ester of 2-pyrrolidone-5- carboxylic acid ("PCA") and is also referred to as lauryl PCA.

Six Franz cells were set up as before (Example 16) except that the heat separated epidermis was installed with the epidermal layer down, i.e., stratum corneum side facing up. Hydration was established by exposing each sample to distilled water overnight. To begin the experiment, the distilled water in the lower chambers was replaced with methylene blue dye solution in all six cells. The upper chambers were filled with distilled water and the cells were observed for about 30 minutes confirming no passage of dye to ensure that no pinhole perforations were present in any of the cells. When none were found, the distilled water in the upper chambers was removed from four of the cells. The other two cells served as distilled water controls. The upper chambers of two of the experimental cells were then filled with CE1 and the other two experimental cells were filled with CE2.

Sonic energy was immediately applied to one of the two CE2 cells. A 25 MHz transducer was used with the frequency sweeping every 0.1 second from 10 MHz to 30 MHz at maximum intensity of ≈0.13 watts/cm^2. After 10-15 minutes of sonic energy applied at a 50% duty cycle, dye flux was visually detected. No dye flux was detected in the other five cells.

Sonic energy was then applied to one of the two cells containing CE1 at the same settings. Dye began to appear in the upper chamber within 5 minutes. Thus, sonic energy together with a chemical enhancer significantly increased the transdermal flux rate of a marker dye through the stratum corneum, as well as reduced the lag time.

Example 20

Effects of Sonic Energy and Chemical Enhancers

Formulations of the two chemical enhancers, CE1 and CE2, were prepared minus the glycerin and these new formulations, designated CE1MG and CE2MG, were tested as before. Water was substituted for glycerin so that the proportions of the other components remained unchanged. Three cells were prepared in modified Franz cells with the epidermal side of the heat separated epidermis samples facing toward the upper side of the chambers. These samples were then hydrated in distilled water for 8 hours. After the hydration step, the distilled water in the lower chambers was replaced with either CE1MG or CE2MG and the upper chamber was filled with the dye solution. Sonic energy was applied to each of the three cells sequentially.

Upon application of pulsed, frequency-modulated sonic energy for a total duration of less than 10 minutes, a significant increase in permeability of the stratum corneum samples was observed. The permeability of the stratum corneum was altered relatively uniformly across the area exposed to both the chemical enhancer and sonic energy. No "pinhole" perforations through which the dye could traverse the stratum corneum were observed. The transdermal flux rate was

instantly controllable by turning the sonic energy on or off. Turning the sonic energy off appeared to instantly reduce the transdermal flux rate such that no dye was visibly being actively transported through the skin sample; presumably the rate was reduced to that of passive diffusion. Turning the sonic energy on again instantly resumed the high level flux rate. The modulated mode appeared to provide a regular pulsatile increase in the transdermal flux rate at the modulated rate. When the sonic energy was set to a constant frequency, the maximum increase in transdermal flux rate for this configuration seemed to occur at around 27 MHz.

Having obtained the same results with all three samples, the cells were then drained of all fluids and flushed with distilled water on both sides of the stratum corneum. The lower chambers were then immediately filled with distilled water and the upper chambers were refilled with dye solution. The cells were observed for 30 minutes. No holes in the stratum corneum samples were observed and no large amount of dye was detected in the lower chambers. A small amount of dye became visible in the lower chambers, probably due to the dye and enhancer trapped in the skin samples from their previous exposures. After an additional 12 hours, the amount of dye detected was still very small.

Example 21

Effects of Sonic Energy and Chemical Enhancers

Perforated Stratum Corneum: Three cells were prepared with heat-separated epidermis samples with the epidermal side facing toward the upper side of the chamber from the same donor as in Example 16. The samples were hydrated for 8 hours and then the distilled water in the lower chambers was replaced with either CE1MG or CE2MG. The upper chambers were then filled with dye solution. Pinhole perforations in the stratum corneum samples permitted dye to leak through the stratum corneum samples into the underlying enhancer containing chambers. Sonic energy was applied. Immediately upon application of the sonic energy, the dye molecules were rapidly pushed through the pores. As shown above, the rapid flux of the dye through the pores was directly and immediately correlated with the application of the sonic energy.

Example 22

Effects of Sonic Energy and Chemical Enhancers

A low cost sonic energy transducer, TDK #NB-58S-01 (TDK Corp.), was tested for its capability to enhance transdermal flux rates. The peak response of this transducer was determined to be about 5.4 MHz with other local peaks occurring at about 7 MHz, 9 MHz, 12.4 MHz, and 16 MHz.

This TDK transducer was then tested at 5.4 MHz for its ability to enhance transdermal flux rate in conjunction with CE1MG. Three cells were set up with the epidermal side facing the lower chamber, then the skin samples were hydrated for 8 hrs. The dye solution was placed in

the lower chamber. The transducer was placed in the upper chamber immersed in CE1MG. Using swept frequencies from 5.3 to 5.6 MHz as the sonic energy excitation, significant quantities of dye moved through the stratum corneum and were detected in the collection well of the cell in 5 minutes. Local heating occurred, with the transducer reaching a temperature of 48°C. In a control using CE1MG without sonic energy, a 24 hour exposure yielded less dye in the collection well than the 5 minute exposure with sonic energy.

This example demonstrates that a low cost, low frequency sonic energy transducer can strikingly affect transdermal flux rate when used in conjunction with an appropriate chemical enhancer. Although higher frequency sonic energy will theoretically concentrate more energy in the stratum corneum, when used with a chemical enhancer, the lower frequency modulated sonic energy can accelerate the transdermal flux rate to make the technology useful and practical.

Example 23

Demonstration of molecule migration across human skin: Tests with the TDK transducer and CE1MG described above were repeated at about 12.4 MHz, one of the highest local resonant peaks for the transducer, with a frequency sweep at a 2 Hz rate from 12.5 to 12.8 MHz and an sonic energy density less than 0.1 W/cm^2. The epidermal side of the heat-separated epidermis was facing down, the dye solution was in the lower chamber, and the enhancer solution and the sonic energy were placed in the upper chamber. Within 5 minutes a significant amount of dye had moved across the stratum corneum into the collection well. Ohmic heating in the transducer was significantly less than with the same transducer being driven at 5.4 MHz, causing an increase in temperature of the chemical enhancer to only about 33°C.

Even at these low efficiency levels, the results obtained with CE1MG and sonic energy from the TDK transducer were remarkable in the monitoring direction. FIGS. 3A and 3B of U.S. Patent No. 5,445,611 show plots of data obtained from three separate cells with the trandermal flux rate measured in the monitoring direction. Even at the 5 minute time point, readily measurable quantities of the dye were present in the chemical enhancer on the outside of the stratum corneum, indicating transport from the epidermal side through the stratum corneum to the "outside" area of the skin sample.

To optimize the use of the sonic energy or the sonic energy/chemical enhancer approach for collecting and monitoring analytes from the body, means for assaying the amount of analyte of interest are required. An assay system that takes multiple readings while the unit is in the process of withdrawing analytes by sonic energy with or without chemical enhancers makes it unnecessary to standardize across a broad population base and normalize for different skin characteristics and flux rates. By plotting two or more data points in time as the analyte concentration in the collection system is increasing, a curve-fitting algorithm can be applied to determine the parameters describing the curve relating analyte withdrawal or flux rate to the point

at which equilibrium is reached, thereby establishing the measure of the interval concentration. The general form of this curve is invariant from one individual to another; only the parameters change. Once these parameters are established, solving for the steady state solution (i.e., time equals infinity) of this function, i.e., when full equilibrium is established, provides the concentration of the analyte within the body. Thus, this approach permits measurements to be made to the desired level of accuracy in the same amount of time for all members of a population regardless of individual variations in skin permeability.

Several existing detection techniques currently exist that are adaptable for this application. See, D.A. Christensen, in 1648 Proceedings of Fiber Optic, Medical and Fluorescent Sensors and Applications 223-26 (1992). One method involves the use of a pair of optical fibers that are positioned close together in an approximately parallel manner. One of the fibers is a source fiber, through which light energy is conducted. The other fiber is a detection fiber connected to a photosensitive diode. When light is conducted through the source fiber, a portion of the light energy, the evanescent wave, is present at the surface of the fiber and a portion of this light energy is collected by the detection fiber. The detection fiber conducts the captured evanescent wave energy to the photosensitive diode which measures it. The fibers are treated with a binder to attract and bind the analyte that is to be measured. As analyte molecules bind to the surface (such as the analyte glucose binding to immobilized lectins such as concanavalin A, or to immobilized anti-glucose antibodies) the amount of evanescent wave coupling between the two fibers is changed and the amount of energy captured by the detection fiber and measured by the diode is changed as well. Several measurements of detected evanescent wave energy over short periods of time support a rapid determination of the parameters describing the equilibrium curve, thus making possible calculation of the concentration of the analyte within the body. The experimental results showing measurable flux within 5 minutes (FIGS. 3A and 3B of U.S. Patent No. 5,445,611) with this system suggest sufficient data for an accurate final reading are collected within 5 minutes.

In its most basic embodiment, a device that can be utilized for the application of sonic energy and collection of analyte comprises an absorbent pad, either of natural or synthetic material, which serves as a reservoir for the chemical enhancer, if used, and for receiving the analyte from the skin surface. The pad or reservoir is held in place, either passively or aided by appropriate fastening means, such as a strap or adhesive tape, on the selected area of skin surface.

An sonic energy transducer is positioned such that the pad or reservoir is between the skin surface and the transducer, and held in place by appropriate means. A power supply is coupled to the transducer and activated by switch means or any other suitable mechanism. The transducer is activated to deliver sonic energy modulated in frequency, phase or intensity, as desired, to deliver the chemical enhancer, if used, from the reservoir through the skin surface followed by collection of the analyte from the skin surface into the reservoir. After the desired fixed or

variable time period, the transducer is deactivated. The pad or reservoir, now containing the analyte of interest, can be removed to quantitate the analyte, for example, by a laboratory utilizing any number of conventional chemical analyses, or by a portable device. Alternately, the mechanism for quantitating the analyte can be build into the device used for collection of the analyte, either as an integral portion of the device or as an attachment. Devices for monitoring an analyte are described in U.S. Patent No. 5,458,140, which is incorporated herein by reference.

Example 24

An alternate method for detection of an analyte, such as glucose, following the sample collection through the porated skin surface as described above, can be achieved through the use of enzymatic means. Several enzymatic methods exist for the measurement of glucose in a biological sample. One method involves oxidizing glucose in the sample with glucose oxidase to generate gluconolactone and hydrogen peroxide. In the presence of a colorless chromogen, the hydrogen peroxide is then converted by peroxidase to water and a colored product.

$$\text{Glucose} \xrightarrow{\text{Glucose Oxidase}} \text{Gluconolactone} + H_2O_2$$

$$2\ H_2O_2 + \text{chromogen} \longrightarrow H_2O + \text{colored product}$$

The intensity of the colored product will be proportional to the amount of glucose in the fluid. This color can be determined through the use of conventional absorbance or reflectance methods. By calibration with known concentrations of glucose, the amount of color can be used to determine the concentration of glucose in the collected analyte. By testing to determine the relationship, one can calculate the concentration of glucose in the blood of the subject. This information can then be used in the same way that the information obtained from a blood glucose test from a finger puncture is used. Results can be available within five to ten minutes.

Example 25

Any system using a visual display or readout of glucose concentration will indicate to a diagnostician or patient the need for administration of insulin or other appropriate medication. In critical care or other situations where constant monitoring is desired and corrective action needs to be taken almost concurrently, the display may be connected with appropriate signal means which triggers the administration of insulin or other medication in an appropriate manner. For

example, there are insulin pumps which are implanted into the peritoneum or other body cavity which can be activated in response to external or internal stimuli. Alternatively, utilizing the enhanced transdermal flux rates possible with micro-poration of the stratum corneum and other techniques described in this invention, an insulin delivery system could be implemented transdermally, with control of the flux rates modulated by the signal from the glucose sensing system. In this manner a complete biomedical control system can be available which not only monitors and/or diagnoses a medical need but simultaneously provides corrective action.

Biomedical control systems of a similar nature could be provided in other situations such as maintaining correct electrolyte balances or administering analgesics in response to a measured analyte parameter such as prostaglandins.

Example 26

Similar to audible sound, sonic waves can undergo reflection, refraction, and absorption when they encounter another medium with dissimilar properties [D. Bommannan et al., 9 Pharm. Res. 559 (1992)]. Reflectors or lenses may be used to focus or otherwise control the distribution of sonic energy in a tissue of interest. For many locations on the human body, a fold of flesh can be found to support this system. For example, an earlobe is a convenient location which would allow use of a reflector or lens to assist in exerting directional control (e.g., "pushing" of analytes or permeants through the porated stratum corneum) similar to what is realized by changing sonic frequency and intensity.

Example 27

Multiple sonic energy transducers may be used to selectively direct the direction of transdermal flux through porated stratum corneum either into the body or from the body. A fold of skin such as an earlobe allow transducers to be located on either side of the fold. The transducers may be energized selectively or in a phased fashion to enhance transdermal flux in the desired direction. An array of transducers or an acoustic circuit may be constructed to use phased

array concepts, similar to those developed for radar and microwave communications systems, to direct and focus the sonic energy into the area of interest.

Example 28

In this example, the procedure of Example 19 is followed with the exception that the heat-separated epidermis samples are first treated with an excimer laser (e.g. model EMG/200 of Lambda Physik; 193 nm wavelength, 14 ns pulsewidth) to ablate the stratum corneum according to the procedure described in U.S. Patent No. 4,775,361, hereby incorporated by reference.

Example 29

In this example, the procedure of Example 19 is followed with the exception that the heat-separated epidermis samples are first treated with 1,1'-diethyl-4,4'-carbocyanine iodide (Aldrich, λ_{max}=703 nm) and then a total of 70 mJ/cm^2/50 ms is delivered to the dye-treated sample with a model TOLD9150 diode laser (Toshiba America Electronic, 30 mW at 690 nm) to ablate the stratum corneum.

Example 30

In this example, the procedure of Example 29 is followed with the exception that the dye is indocyanine green (Sigma cat. no. I-2633; λ_{max}=775 nm) and the laser is a model Diolite 800-50 (LiCONiX, 50 mW at 780 nm).

Example 31

In this example, the procedure of Example 29 is followed with the exception that the dye is methylene blue and the laser is a model SDL-8630 (SDL Inc.; 500 mW at 670 nm).

Example 32

In this example, the procedure of Example 29 is followed with the exception that the dye is contained in a solution comprising a permeation enhancer, e.g. CE1.

Example 33

In this example, the procedure of Example 29 is followed with the exception that the dye and enhancer-containing solution are delivered to the stratum corneum with the aid of exposure to ultrasound.

Example 34

In this example, the procedure of Example 31 is followed with the exception that the pulsed light source is a short arc lamp emitting over the broad range of 400 to 1100 nm but having a bandpass filter placed in the system to limit the output to the wavelength region of about 650 to 700 nm.

Example 35

In this example, the procedure of Example 19 is followed with the exception that the heat-separated epidermis samples are first punctured with a microlancet (Becton Dickinson) calibrated to produce a micropore in the stratum corneum without reaching the underlying tissue.

Example 36

In this example, the procedure of Example 19 is followed with the exception that the heat-separated epidermis samples are first treated with focused sonic energy in the range of 70-480 $mJ/cm^2/50$ ms to ablate the stratum corneum.

Example 37

In this example, the procedure of Example 19 is followed with the exception that the stratum corneum is first punctured hydraulically with a high pressure jet of fluid to form a micropore of up to about 100 μm diameter.

Example 38

In this example, the procedure of Example 19 is followed with the exception that the stratum corneum is first punctured with short pulses of electricity to form a micropore of up to about 100 μm diameter.

Example 39

Acoustic Streaming

A new mechanism and application of sonic energy in the delivering of therapeutic substances into the body and/or harvesting fluids from within the body into an external reservoir through micro-porations formed in the stratum corneum layer will now be described. An additional aspect of this invention is the utilization of sonic energy to create an acoustic streaming effect on the fluids flowing around and between the intact cells in the epidermis and dermis of the human skin. Acoustic streaming is a well documented mode by which sonic energy can interact with a fluid medium. Nyborg, Physical Acoustics Principles and Methods, p. 265-331, Vol II-Part B, Academic Press, 1965. The first theoretical analysis of acoustic streaming phenomenon was given by Rayleigh (1884, 1945). In an extensive treatment of the subject, Longuet-Higgins (1953-1960) has given a result applicable to two dimensional flow that results in the near vicinity of any vibrating cylindrical surface. A three dimensional approximation for an arbitrary surface was developed by Nyborg (1958). As described by Fairbanks et al., 1975 Ultrasonics Symposium Proceedings, IEEE Cat. #75, CHO 994-4SU, sonic energy, and the acoustic streaming phenomenon can be of great utility in accelerating the flux of a fluid through a porous medium, showing measurable increases in the flux rates by up to 50 times that possible passively or with only pressure gradients being applied.

All previous transdermal delivery or extraction efforts utilizing ultrasound have focused on methods of interaction between the sonic energy and the skin tissues designed to permeabilize the stratum corneum layer. The exact mode of interaction involved has been hypothesized to be due exclusively to the local elevation of the temperature in the SC layer, and the resultant melting of the lipid domains in the intercellular spaces between the corneocytes.

Srinivasan et al. Other researchers have suggested that micro-cavitations and or shearing of the structures in the stratum corneum opens up channels through which fluids may flow more readily. In general, the design of the sonic systems for the enhancement of transdermal flux rates has been based on the early realization that the application of an existing therapeutic ultrasound unit designed to produce a "deep-heating" effect on the subject, when used in conjunction with a topical application of a gelled or liquid preparation containing the drug to be delivered into the body, could produce a quantifiable increase in the flux rate of the drug into the body. in the context of the method taught herein to create micro-pores in this barrier layer, the use of sonic energy may now be thought of in a totally new and different sense than the classically defined concepts of sonophoresis.

Based on the experimental discovery mentioned in U.S. patents 5,458,140 and 5,445,611 that when a small hole existed or was created in the stratum corneum (SC) in the Franz cells used in the in vitro studies, that the application of an appropriately driven ultrasonic transducer to the fluid reservoir on either side of the porated SC sample, an "acoustic streaming" event could be generated wherein large flux rates of fluid where capable of being pumped through this porated membrane.

With the method taught herein to create the controlled micro-porations in the stratum corneum layer in the living subject's skin, the application of the fluid streaming mode of sonic/fluid interaction to the induction of fluid into or out of the body may now be practically explored. For example, clinical studies have shown that by making a series of four 80 μm diameter micro-pores in a 400 μm square, and then applying a mild (10 to 12 inches of Hg) suction to this area, an average of abouit 1 μl of interstitial fluid can be induced to leave the body for external collection in an external chamber. By adding a small, low power sonic transducer to this system, configured such that it actively generates inwardly converging concentric circular pressure waves in the 2 to 6 mm of tissue surrounding the poration site, it has been demonstrated that this ISF flux rate can be increased by 50%.

By relieving ourselves of the desire to create some form of direct absorption of sonic energy in the skin tissues (as required to generate heating), frequencies of sonic energy can be

determined for which the skin tissues are virtually transparent, that is at the very low frequency region of 1 kHz to 500 KHz. Even at some of the lowest frequencies tested, significant acoustic streaming effects could be observed by using a micro-scope to watch an in vivo test wherein the subject's skin was micro-porated and ISF was induced to exit the body an pool on the surface of the skin. Energizing the sonic transducer showed dramatic visual indications of the amount of acoustic streaming as small pieces of particulate matter were carried along with the ISF as it swirled about. Typical magnitude of motion exhibited can be described as follows: for a 3 mm diameter circular pool of ISF on the surface of the skin, a single visual particle could be seen to be completing roughly 3 complete orbits per second. This equates to a linear fluid velocity of more than 2.5 mm/second. All of this action was demonstrated with sonic power levels into the tissues of less than 100 mW/cm2.

While one can easily view the top surface of the skin, and the fluidic activity thereon, assessing what is taking place dynamically within the skin tissue layers in response to the coupling into these tissues of sonic energy is much more difficult. One can assume, that if such large fluid velocities (e.g. >2.5 mm/S) may be so easily induced on the surface, then some noticeable increase in the fluid flow in the intercellular channels present in the viable dermal tissues could also be realized in response to this sonic energy input. Currently, an increase in harvested ISF through a given set of microporations when a low frequency sonic energy was applied to the area in a circle surrounding the poration sites has been quantified. In this experiment, an ISF harvesting technique based solely on a mild suction (10 to 12 inches of HG) was alternated with using the exact same apparatus, but with the sonic transducer engaged. Over a series of 10 two-minute harvesting periods, five with mere suction and five with both suction and sonic energy active, it was observed that by activating the sonic source roughly 50% more ISF was collectable in the same time period. These data are shown in FIG. 30. This increase in ISF flux rate was realized with no reported increase in sensation from the test subject due to the sonic energy. The apparatus used for this experiment is illustrated in FIGS. 31-33. The transducer assembly in FIGS. 31-33 is comprised of a thich walled cylinder of piezo-electric material, with an internal diameter of roughly 8 mm and a wall thickness of 4 mm. The cylinder has been polarized such that when

an electrical field is applied across the metalized surfaces of the outer diameter and inner diameter, the thickness of the wall of the cylinder expands or contracts in response to the field polarity. In practice, this configuration results in a device which rapidly squeezes the tissue which has been suctioned into the central hole, causing an inward radial acoustic streaming effect on those fluids present in these tissues. This inward acoustic streaming is responsible for bringing more ISF to the location of the micro-porations in the center of the hole, where it can leave the body for external collection.

A similar device shown in FIG. 34A-B was built and tested and produced similar initial results. In the FIG. 34A-B version, an ultrasonic transducer built by Zevex, Inc. Salt Lake City, Utah, was modified by having a spatulate extension added to the sonic horn. A 4 mm hole was placed in the 0.5 mm thick spatulate end of this extension. When activated, the principle motion is longitudinal along the length of the spatula, resulting in essentially a rapid back and forth motion. The physical perturbation of the metalic spatula casued by the placement of the 4 mm hole, results in a very active, but chaotic, large displacement behavior at this point. In use, the skin of the subject was suctioned up into this hole, and the sonic energy was then cunductined into the skin in a fashion similar to that illustrated in FIG. 33.

The novel aspect of this new application of ultrasound lies in the following basic areas:

1. The function of the ultrasound is no longer needed to be focused on permeabilizing the SC barrier membrane as taught by Langer, Kost, Bommannan and others.

2. A much lower frequency system can be utilized which has very little absorption in the skin tissues, yet can still create the fluidic streaming phenomenon desired within the intercellular passageways between the epidermal cells which contain the interstitial fluid.

3. The mode of interaction with the tissues and fluids therein, is the so-called "streaming" mode, recognized in the sonic literature as a unique and different mode than the classical vibrational interactions capable of shearing cell membranes and accelerating the passive diffusion process.

By optimizing the geometric configuration, frequency, power and modulations applied to the sonic transducer, it has been shown that significant increases in the fluid flux through the

porated skin sites can be achieved. The optimization of these parameters is designed to exploit the non-linearities g verning the fluid flow relationships in this microscopically scaled environment. Using frequencies under 200 kHz, large fluidic effects can be observed, without any detectable heating or other negative tissue interactions. The sonic power levels required to produce these measurable effects are very low, with average power levels typically under 100 milliwatts/cm2.

Therefore, the above examples are but representative of systems which may be employed in the utilization of ultrasound or ultrasound and chemical enhancers in the collection and quantification of analytes for diagnostic purposes and for the transdermal delivery of permeants. The invention is directed to the discovery that the poration of the stratum corneum followed by the proper use of ultrasound, particularly when accompanied with the use of chemical enhancers, enables the noninvasive or minimally invasive transdermal determination of analytes or delivery of permeants. However, the invention is not limited only to the specific illustrations. There are numerous poration techniques and enhancer systems, some of which may function better than another, for detection and withdrawn of certain analytes or delivery of permeants through the stratum corneum. However, within the guidelines presented herein, a certain amount of experimentation to obtain optimal poration, enhancers, or optimal time, intensity and frequency of applied ultrasound, as well as modulation of frequency, amplitude and phase of applied ultrasound can be readily carried out by those skilled in the art. Therefore, the invention is limited in scope only by the following claims and functional equivalents thereof.

5

CLAIMS

We claim:

1. A method for monitoring the concentration of an analyte in an individual's body comprising the steps of enhancing the permeability of the stratum corneum of a selected area of the individual's body surface to the analyte by means of

(a) porating the stratum corneum of said selected area by means that form a micro-pore in said stratum corneum without causing serious damage to the underlying tissues, thereby reducing the barrier properties of said stratum corneum to the withdrawal of said analyte;

(b) collecting a selected amount of the analyte; and

(c) quantitating the analyte collected.

2. The method of to Claim 1 further comprising applying sonic energy to said porated selected area at a frequency in the range of about 5 kHz to 100 MHz, wherein said sonic energy is modulated by means of a member selected from the group consisting of frequency modulation, amplitude modulation, phase modulation, and combinations thereof.

3. The method of to Claim 2 further comprising contacting the selected area of the individual's body with a chemical enhancer with the application of the sonic energy to further enhance analyte withdrawal.

4. The method of Claims 1, 2, 3, or 4 wherein said porating of said stratum corneum in said selected area is accomplished by means selected from the group consisting of (a) ablating the stratum corneum by contacting a selected area, up to about 1000 μm across, of said stratum corneum with a heat source such that the temperature of tissue-bound water and other vaporizable substances in said selected area is elevated above the vaporization point of said water and other vaporizable substances thereby removing the stratum corneum in said selected area; (b) puncturing said stratum corneum with a micro-lancet calibrated to form a micropore of up to about 1000 μm

in diameter; (d) ablating the stratum corneum by focusing a tightly focused beam of sonic energy onto said stratum corneum; (d) hydraulically puncturing said stratum corneum with a high pressure jet of fluid to form a micropore of up to about 1000 μm in diameter and (e) puncturing said stratum corneum with short pulses of electricity to form a micropore of up to about 1000 μm in diameter.

5. The method of Claim 4 wherein said porating is accomplished by contacting said selected area, up to about 1000 μm across, of said stratum corneum with a heat source such that the temperature of the tissue-bound water and other vaporizable substances in said selected area is elevated above the vaporization point of said water and other vaporizable substances thereby removing the stratum corneum in said selected area.

6. The method of Claim 5 comprising treating at least said selected area with an effective amount of a dye that exhibits strong absorption over the emission range of a pulsed light source and focusing the output of a series of pulses from said pulsed light source onto said dye such that said dye is heated sufficiently to conductively transfer heat to said stratum corneum to elevate the temperature of tissue-bound water and other vaporizable substances in said selected area above the vaporization point of said water and other vaporizable substances, wherein said dye functions as a heat source.

7. The method of Claim 6 wherein said pulsed light source emits at a wavelength that is not significantly absorbed by skin.

8. The method of Claim 7 wherein said pulsed light source is a laser diode emitting in the range of about 630 to 1550 nm.

9. The method of Claim 7 wherein said pulsed light source is a laser diode pumped optical parametric oscillator emitting in the range of about 700 and 3000 nm.

10. The method of Claim 6 wherein said pulsed light source is a member selected from the group consisting of arc lamps, incandescent lamps, and light emitting diodes.

11. The method of claim 6 further comprising providing a sensing system for determining when the barrier properties of the stratum corneum have been surmounted.

12. The method of claim 11 wherein said sensing system comprises light collection means for receiving light reflected from said selected area and focusing said reflected light on a photodiode, a photodiode for receiving said focused light and sending a signal to a controller wherein said signal indicates a quality of said reflected light, and a controller coupled to said photodiode and to said pulsed light source for receiving said signal and for shutting off said pulsed light source when a preselected signal is received.

13. The method of claim 6 further comprising cooling said selected area of stratum corneum and adjacent skin tissues with cooling means such that said selected area and adjacent skin tissues are in a selected precooled, steady state, condition prior to poration.

14. The method of claim 13 wherein said cooling means comprises a Peltier device.

15. The method of claim 6 wherein said ablating results in exudation of interstitial fluid and said analyte is collected in a selected amount of said interstitial fluid.

16. The method of claim 15 further comprising, after said selected amount of interstitial fluid is collected, sealing said micropore by applying an effective amount of energy from said pulsed light source such that interstitial fluid remaining in said micropore is caused to coagulate.

17. The method of claim 15 further comprising applying a vacuum to said porated selected area of stratum corneum for enhancing exudation of interstitial fluid.

18. The method of claim 6 further comprising, prior to porating said stratum corneum, illuminating at least said selected area with unfocused light from said pulsed light source such that said selected area illuminated with said light is sterilized.

19. The method of claim 5 comprising contacting said selected area with a metallic wire, wherein said metallic wire functions as a heat source, such that the temperature of said selected area is raised from ambient skin temperature to greater than 100°C within about 10 to 50 ms and then returning the temperature of said selected area to approximately ambient skin temperature within about 30 to 50 ms and wherein a cycle of raising the temperature and returning to ambient skin temperature is repeated a number of time effective for reducing the barrier properties of the stratum corneum.

20. The method of claim 19 wherein said returning to approximately ambient skin temperature is carried out by withdrawing said wire from contact with the stratum corneum.

21. The method of claim 20 further comprising providing means for monitoring electrical impedance between said wire and said individual's body through said selected area of stratum corneum and adjacent skin tissues and means for advancing the position of said wire such that as said ablation occurs with a concomitant reduction in resistance, wherein said advancing means advances the wire such that the wire is in contact with the stratum corneum during heating of the wire.

22. The method of claim 21 further comprising means for withdrawing said wire from contact with the stratum corneum, wherein said monitoring means is capable of detecting a change in impedance associated with contacting an epidermal layer underlying the stratum corneum and sending a signal to said withdrawing means to withdrawn said wire from contact with the stratum corneum.

23. The method of claim 20 wherein said metallic wire is heated by an ohmic heating element.

24. The method of claim 20 wherein said metallic wire is formed such that it contains a current loop having a high resistance point and the temperature of said high resistance point is modulated by passing a modulated electrical current through said current loop.

25. The method of claim 20 wherein said metallic wire is positioned in a modulatable alternating magnetic field of an excitation coil such that energizing the excitation coil with alternating current produces eddy currents suffient to heat the wire by internal ohmic losses.

26. The method of Claim 4 wherein said poration is accomplished by puncturing said stratum corneum with a micro-lancet calibrated to form a micropore of up to about 1000 μm in diameter.

27. The method of Claim 4 wherein said poration is accomplished by ablating the stratum corneum by focusing a tightly focused beam of sonic energy onto said stratum corneum.

28. The method of Claim 4 wherein said poration is accomplished by hydraulically puncturing said stratum corneum with a high pressure jet of fluid to form a micropore of up to about 1000 μm in diameter.

29. The method of Claim 4 wherein said poration is accomplished by puncturing said stratum corneum with short pulses of electricity to form a micropore of up to about 1000 μm in diameter.

30. The method of Claim 4, 6, or 19 wherein the analyte is glucose.

31. The method of claim 30 wherein the glucose is quantitated by means of a colorimetric assay with glucose oxidase or an electro-chemical biosensor.

32. A method for enhancing the transdermal flux rate of an active permeant into a selected area of an individual's body comprising the steps of enhancing the permeability of the stratum corneum layer of said selected area of the individual's body surface to said active permeant by means of

(a) porating the stratum corneum of said selected area by means that form a micro-pore in said stratum corneum without causing serious damage to the underlying tissues and thereby reduce the barrier properties of said stratum corneum to the flux of said active permeant; and

(b) contacting the porated selected area with a composition comprising an effective amount of said permeant such that the flux of said permeant into the body is enhanced.

33. The method of claim 32 further comprising applying sonic energy to said porated selected area for a time and at an intensity and a frequency effective to create a fluid streaming effect and thereby enhance the transdermal flux rate of said permeant into the body.

34. The method of claim 33 wherein said sonic energy is applied to said porated selected area at a frequency in the range of about 5 kHz to 100 MHz, wherein said sonic energy is modulated by means of a member selected from the group consisting of frequency modulation, amplitude modulation, phase modulation, and combinations thereof.

35. The method of to Claim 34 further comprising contacting the selected area of the individual's body with a chemical enhancer with the application of the sonic energy to further the flux of said permeant into said individual's body.

36. The method of Claims 32, 33, 34, or 35 wherein said porating of said stratum corneum in said selected area is accomplished by means selected from the group consisting of (a) ablating the stratum corneum by contacting a selected area, up to about 1000 μm across, of said stratum corneum with a heat source such that the temperature of tissue-bound water and other vaporizable substances in said selected area is elevated above the vaporization point of said water and other

vaporizable substances thereby removing the stratum corneum in said selected area; (b) puncturing said stratum corneum with a micro-lancet calibrated to form a micropore of up to about 1000 μm in diameter; (d) ablating the stratum corneum by focusing a tightly focused beam of sonic energy onto said stratum corneum; (d) hydraulically puncturing said stratum corneum with a high pressure jet of fluid to form a micropore of up to about 1000 μm in diameter and (e) puncturing said stratum corneum with short pulses of electricity to form a micropore of up to about 1000 μm in diameter.

37. The method of Claim 36 wherein said porating is accomplished by contacting said selected area, up to about 1000 µm across, of said stratum corneum with a heat source such that the temperature tissue-bound water and other vaporizable substances in said selected area is elevated above the vaporization point of said water and other vaporizable substances thereby removing the stratum corneum in said selected area.

38. The method of claim 37 comprising treating at least said selected area with an effective amount of a dye that exhibits strong absorption over the emission range of a pulsed light source and focusing the output of a series of pulses from said pulsed light source onto said dye such that said dye is heated sufficiently to conductively transfer heat to said stratum corneum to elevate the temperature of tissue-bound water and other vaporizable substances in said selected area above the vaporization point of said water and other vaporizable substances, wherein said dye functions as a heat source.

39. The method of Claim 38 wherein said pulsed light source emits at a wavelength that is not significantly absorbed by skin.

40. The method of Claim 39 wherein said pulsed light source is a laser diode emitting in the range of about 630 to 1550 nm.

41. The method of Claim 39 wherein said pulsed light source is a laser diode pumped optical parametric oscillator emitting in the range of about 700 and 3000 nm.

42. The method of Claim 39 wherein said pulsed light source is a member selected from the group consisting of arc lamps, incandescent lamps, and light emitting diodes.

43. The method of claim 38 further comprising providing a sensing system for determining when the barrier properties of the stratum corneum have been surmounted.

44. The method of claim 43 wherein said sensing system comprises light collection means for receiving light reflected from said selected area and focusing said reflected light on a photodiode, a photodiode for receiving said focused light and sending a signal to a controller wherein said signal indicates a quality of said reflected light, and a controller coupled to said photodiode and to said pulsed light source for receiving said signal and for shutting off said pulsed light source when a preselected signal is received.

45. The method of claim 38 further comprising cooling said selected area of stratum corneum and adjacent skin tissues with cooling means such that said selected area and adjacent skin tissues are in a selected precooled, steady state, condition prior to poration.

46. The method of claim 45 wherein said cooling means comprises a Peltier device.

47. The method of claim 38 further comprising, prior to porating said stratum corneum, illuminating at least said selected area with unfocused light from said pulsed light source such that said selected area illuminated with said light is sterilized.

48. The method of claim 37 comprising contacting said selected area with a metallic wire, wherein said wire functions as a heat source, such that the temperature of said selected area is

raised from ambient skin temperature to greater than 100°C within about 10 to 50 ms and then returning the temperature of said selected area to approximately ambient skin temperature within about 30 to 50 ms and wherein a cycle of raising the temperature and returning to ambient skin temperature is repeated a number of time effective for reducing the barrier properties of the stratum corneum.

49. The method of claim 48 wherein said returning to approximately ambient skin temperature is carried out by withdrawing said wire from contact with the stratum corneum.

50. The method of claim 49 further comprising providing means for monitoring electrical impedance between said wire and said individual's body through said selected area of stratum corneum and adjacent skin tissues and means for advancing the position of said wire such that as said ablation occurs with aconcomitant reduction in resistance, wherein said advancing means advances the wire such that the wire is in contact with the stratum corneum during heating of the wire.

51. The method of claim 50 further comprising means for withdrawing said wire from contact with the stratum corneum, wherein said monitoring means is capable of detecting a change in impedance associated with contacting an epidermal layer underlying the stratum corneum and sending a signal to said withdrawing means to withdrawn said wire from contact with the stratum corneum.

52. The method of claim 49 wherein said metallic wire is heated by an ohmic heating element.

53. The method of claim 49 wherein said metallic wire is formed such that it contains a current loop having a high resistance point and the temperature of said high resistance point is modulated by passing a modulated electrical current through said current loop.

54. The method of claim 49 wherein said metallic wire is positioned in a modulatable alternating magnetic field of an excitation coil such that energizing the excitation coil with alternating current produces eddy currents suffient to heat the wire by internal ohmic losses.

55. The method of Claim 36 wherein said poration is accomplished by puncturing said stratum corneum with a micro-lancet calibrated to form a micropore of up to about 1000 μm in diameter.

56. The method of Claim 36 wherein said poration is accomplished by ablating the stratum corneum by focusing a tightly focused beam of sonic energy onto said stratum corneum.

57. The method of Claim 36 wherein said poration is accomplished by hydraulically puncturing said stratum corneum with a high pressure jet of fluid to form a micropore of up to about 1000 μm in diameter.

58. The method of Claim 36 wherein said poration is accomplished by puncturing said stratum corneum with short pulses of electricity to form a micropore of up to about 1000 μm in diameter.

59. A method for appyling a tatoo to a selected area of skin on an individual's body surface comprising the steps of:

(a) porating the stratum corneum of said selected area by means that form a micro-pore in said stratum corneum without causing serious damage to the underlying tissues and thereby reduce the barrier properties of said stratum corneum to the flux of said active permeant; and

(b) contacting the porated selected area with a composition comprising an effective amount of a tattoing ink such that the flux of said ink into the body is enhanced.

60. A method for reducing a temporal delay in diffusion of an analyte from blood of an individual to said individual's interstitial fluid in a selected area of skin comprising applying means for cooling to said selected area of skin.

61. A method for reducing evaporation of interstitial fluid and the vapor pressure thereof, wherein said interstitial fluid is being collected from a micropore in a selected area of stratum corneum of an individual's skin, comprising applying means for cooling to said selected area of skin.

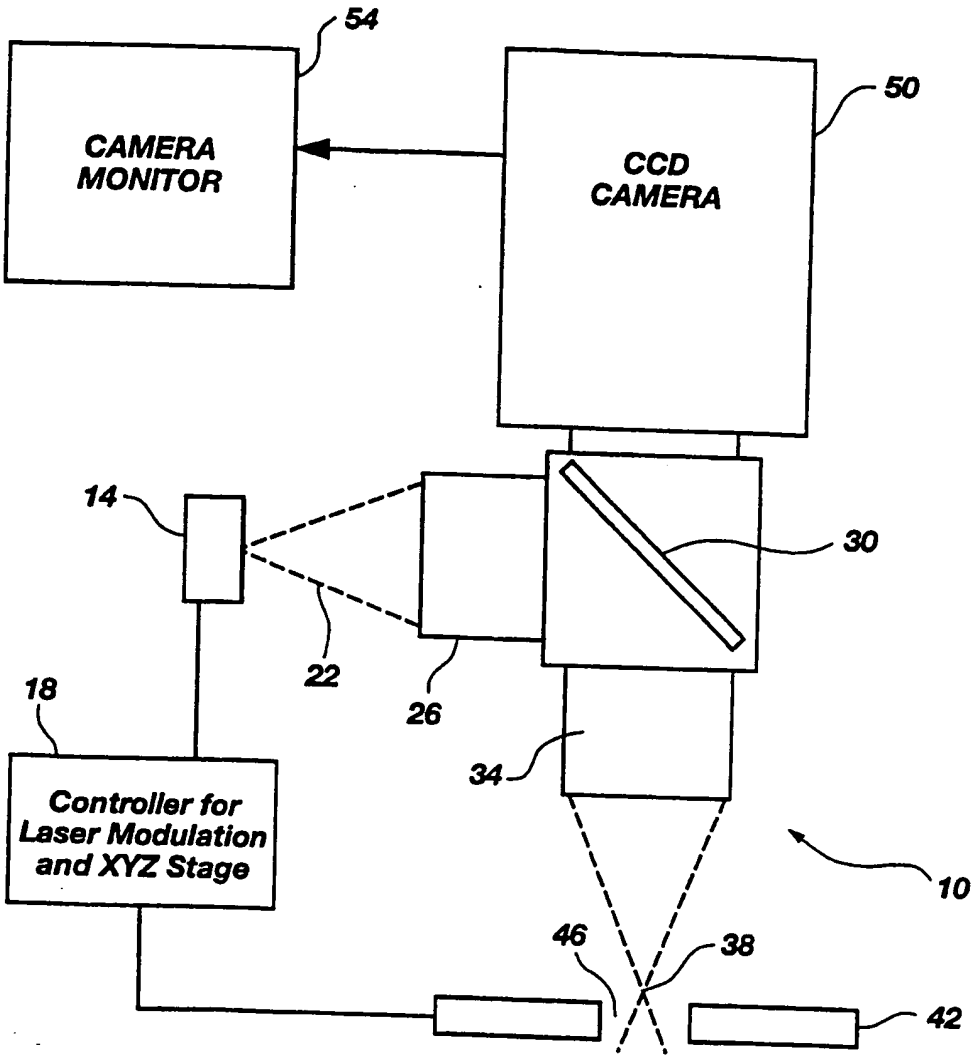

54
CAMERA MONITOR
50
CCD CAMERA
14
30
22
26
18
34
Controller for Laser Modulation and XYZ Stage
10
46
38
42

Fig. 1

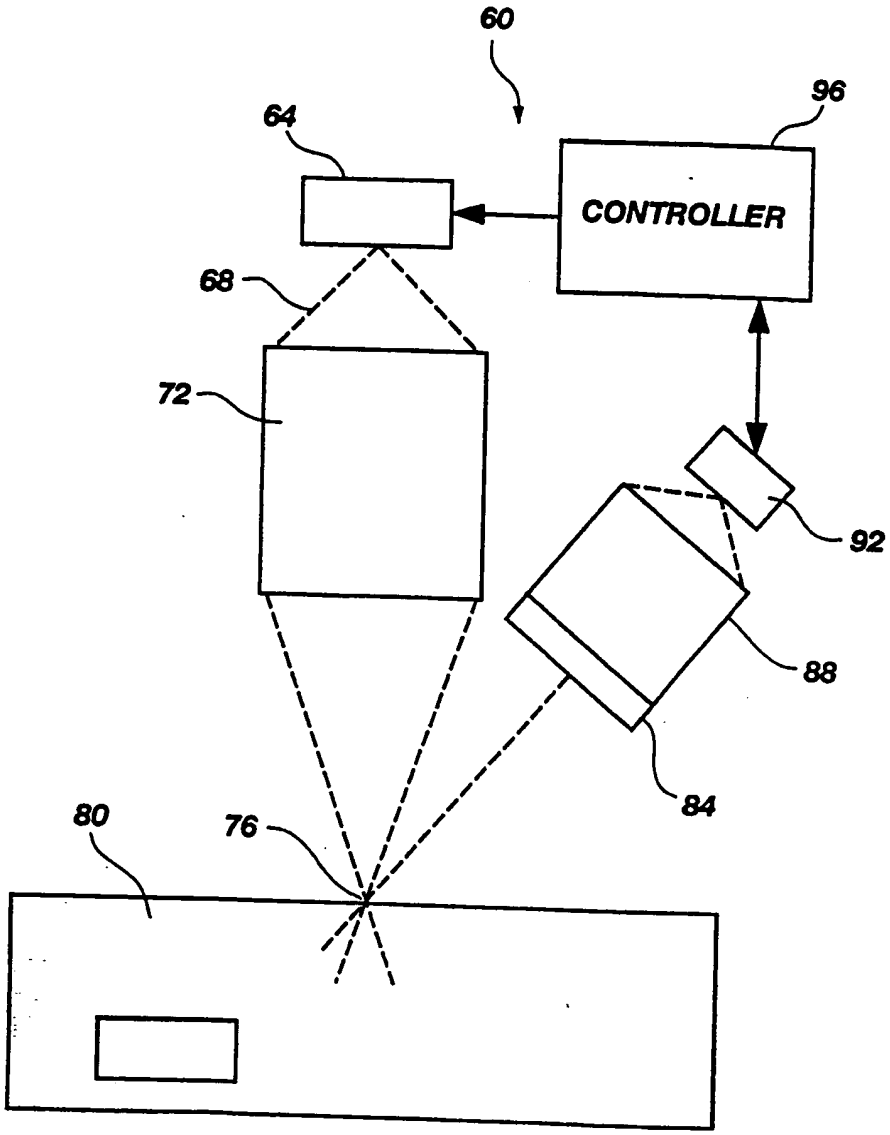


Fig. 2

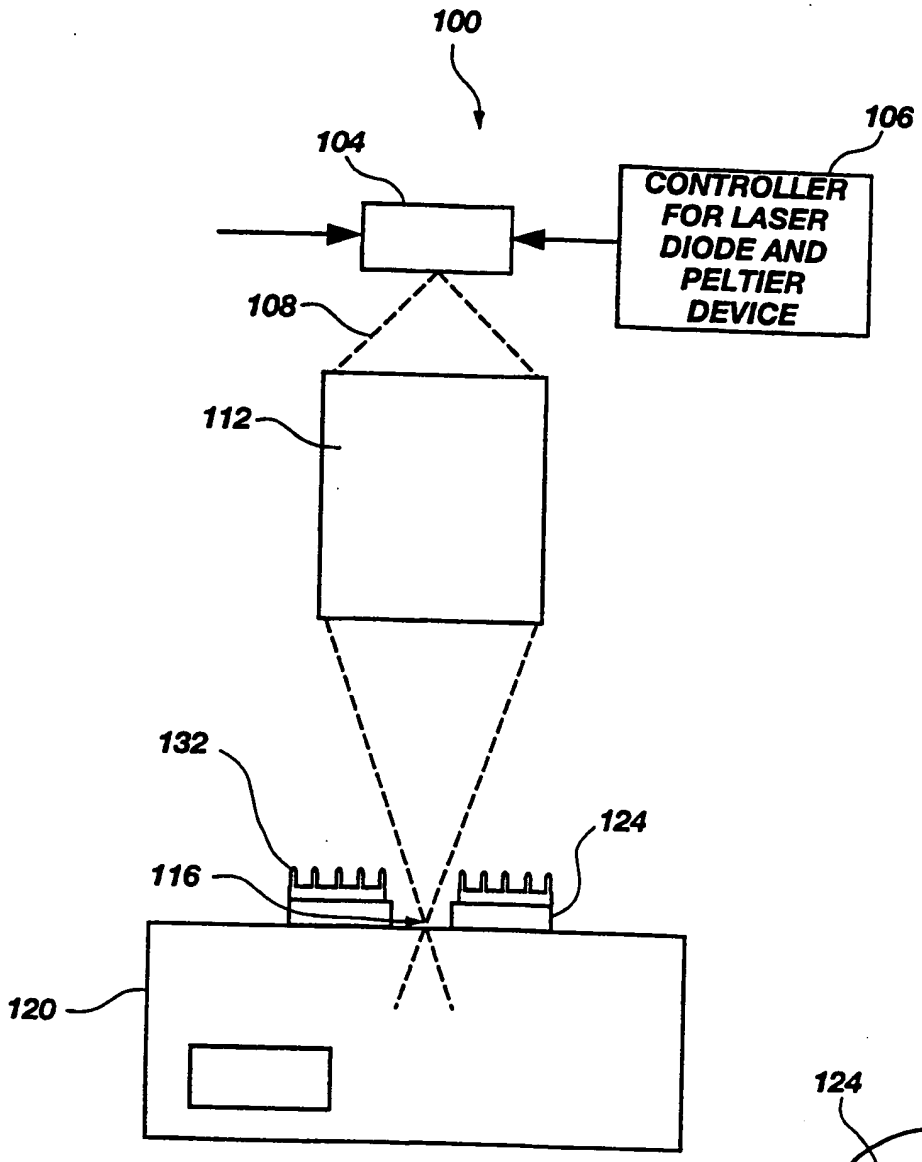
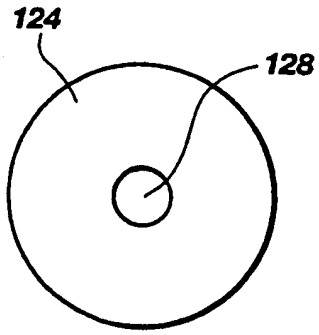

100
104
106
CONTROLLER FOR LASER DIODE AND PELTIER DEVICE
108
112
132
124
116
120

Fig. 3A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128

Fig. 3B

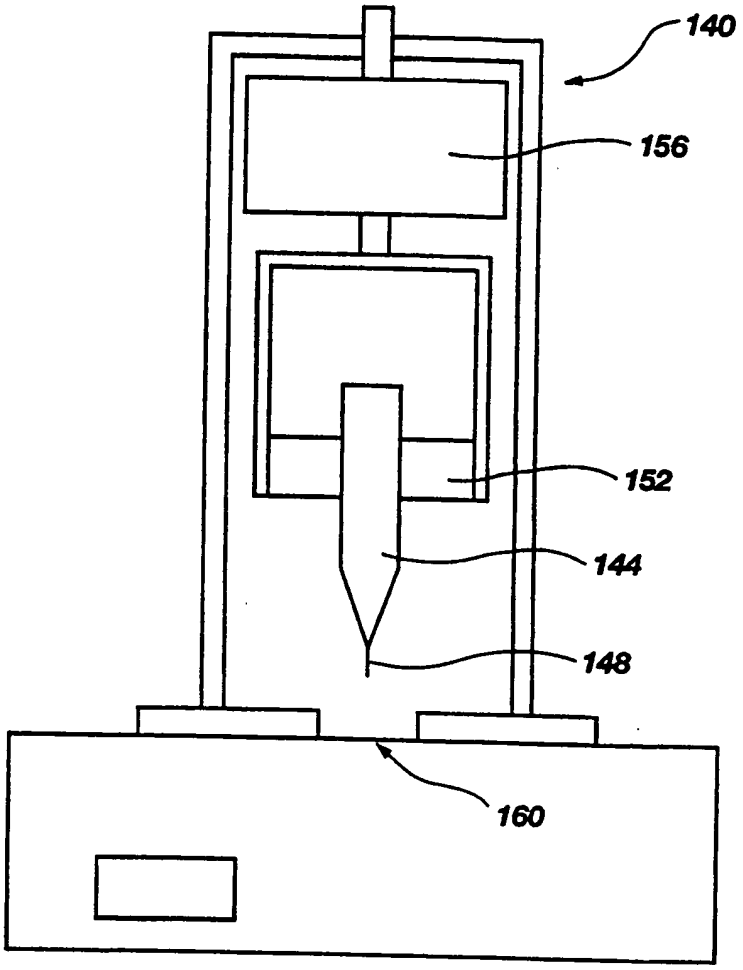

Fig. 4

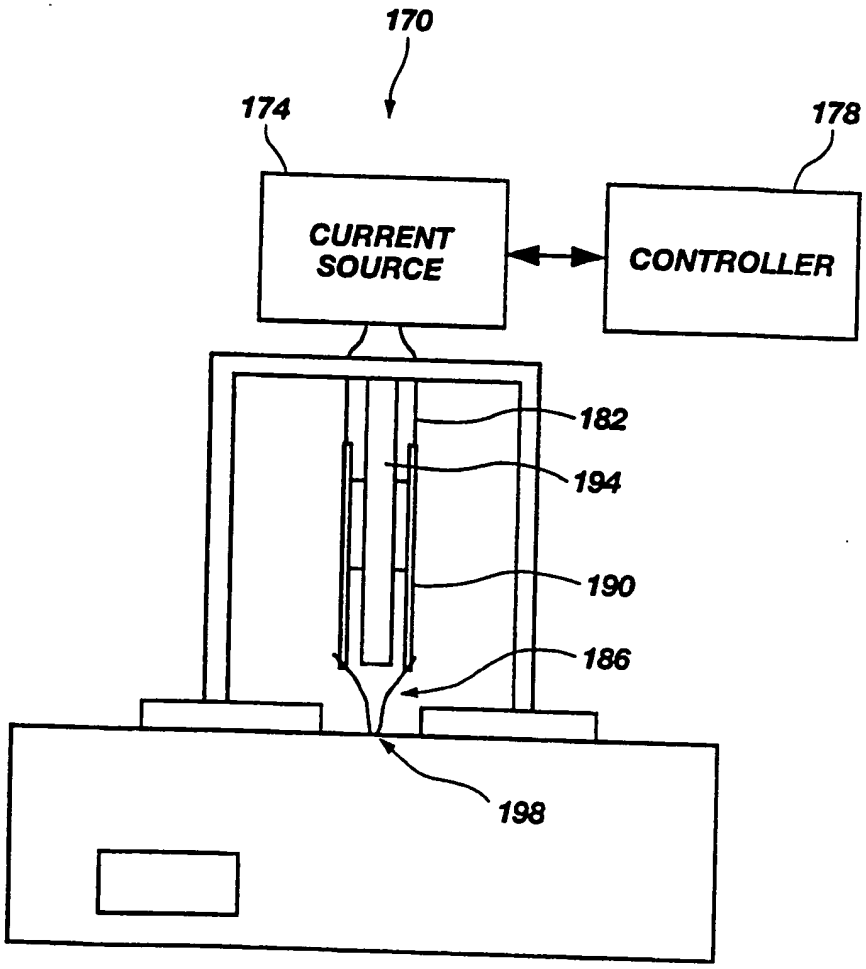

Fig. 5

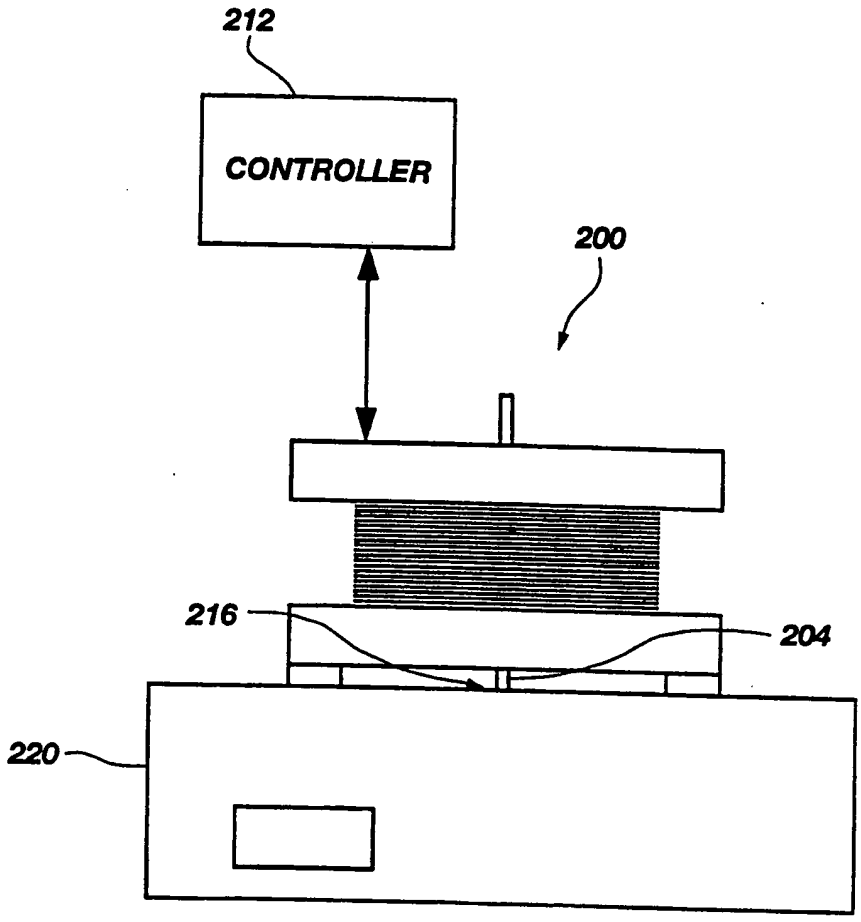

Fig. 6

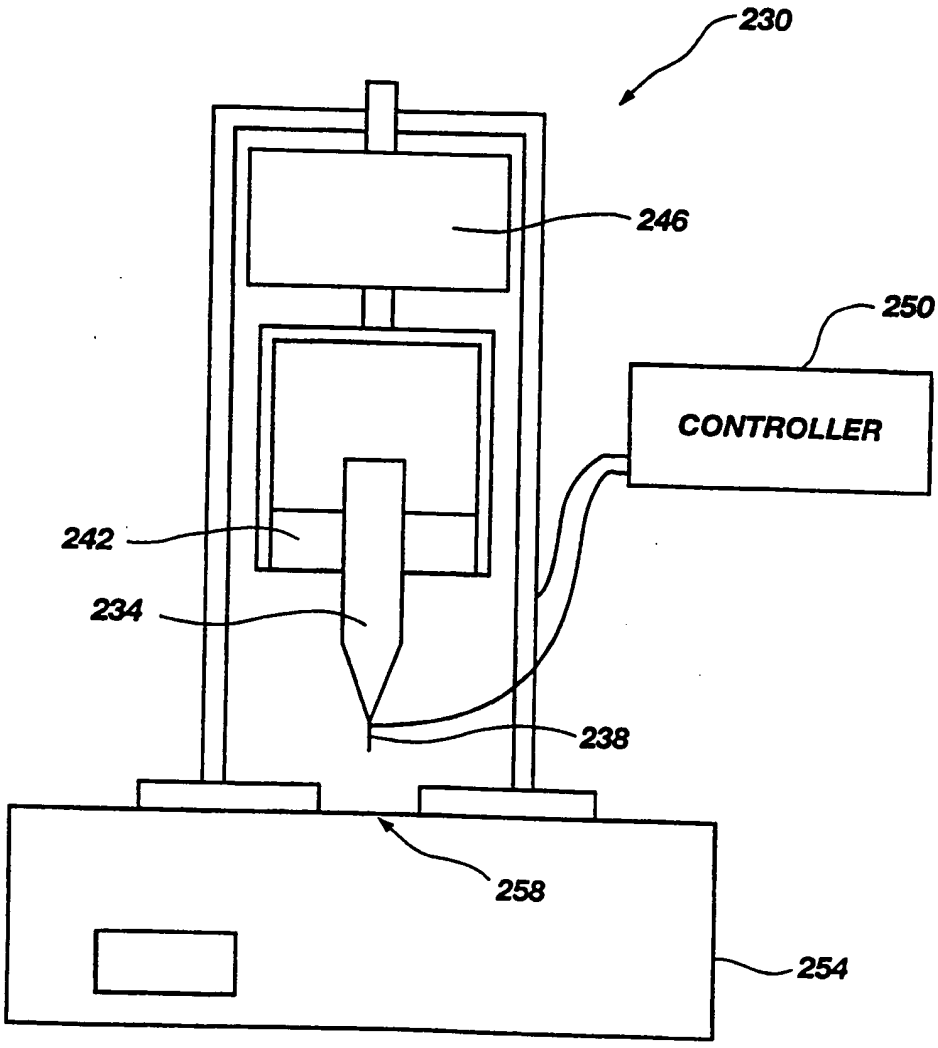

Fig. 7

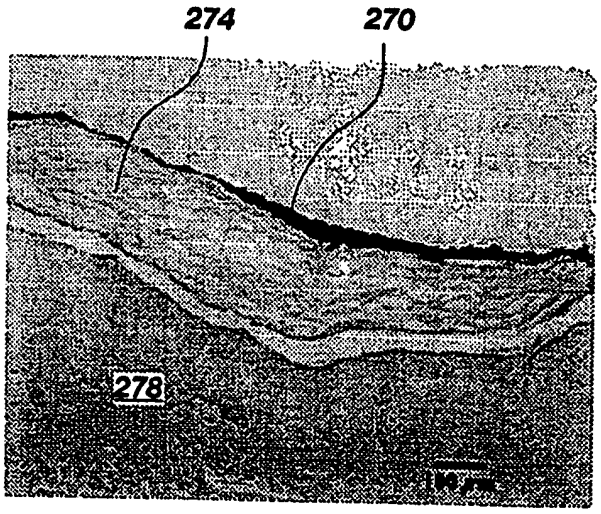

Fig. 8A

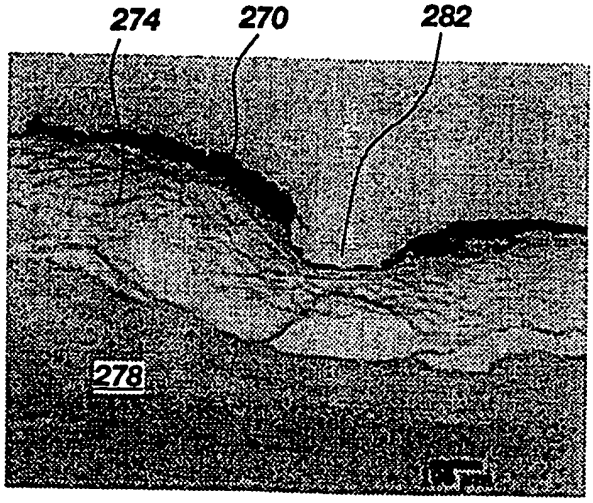

Fig. 8B

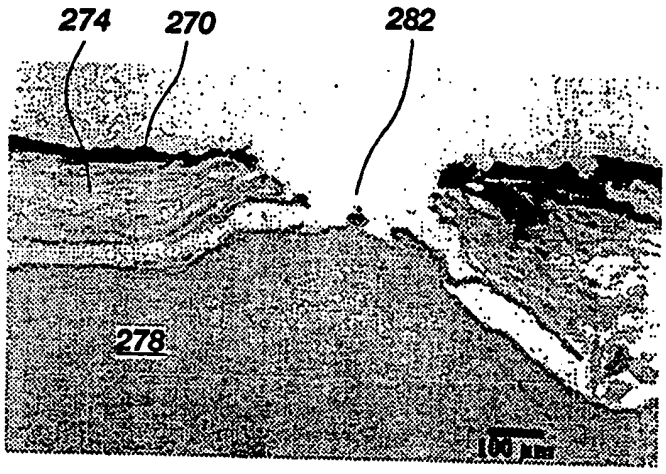

Fig. 8C

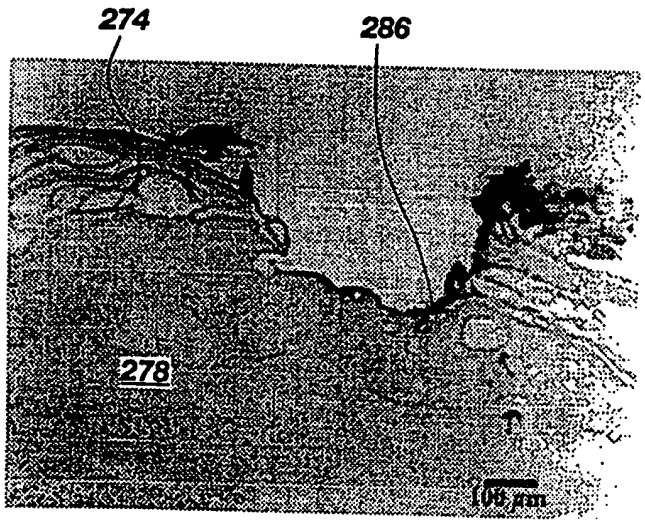

Fig. 8D

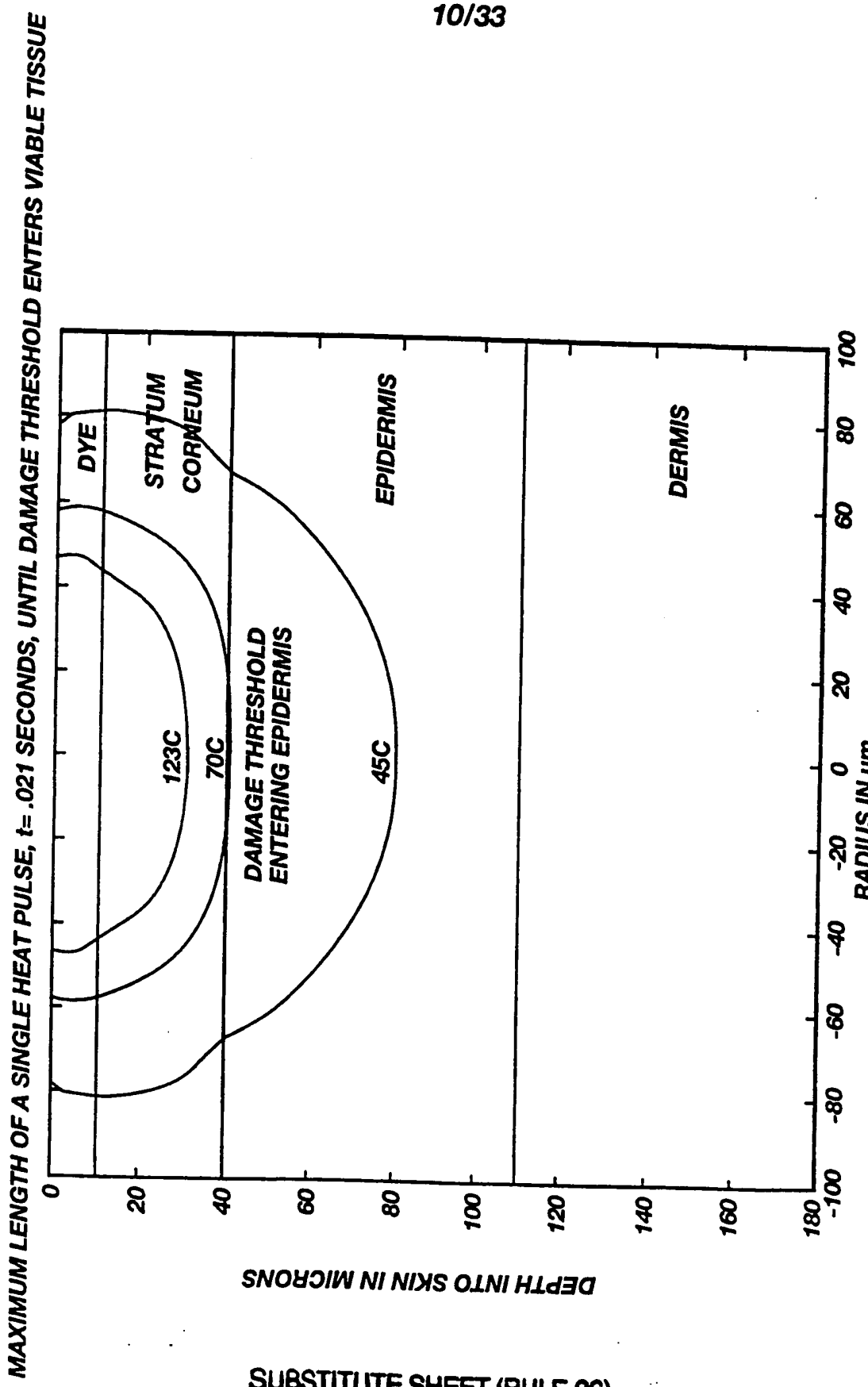
MAXIMUM LENGTH OF A SINGLE HEAT PULSE, t= .021 SECONDS, UNTIL DAMAGE THRESHOLD ENTERS VIABLE TISSUE
DYE
STRATUM CORNEUM
EPIDERMIS
DERMIS
123C
70C
DAMAGE THRESHOLD ENTERING EPIDERMIS
45C
0
20
40
60
80
100
120
140
160
180
-100
-80
-60
-40
-20
0
20
40
60
80
100
RADIUS IN µm
DEPTH INTO SKIN IN MICRONS

Fig. 9

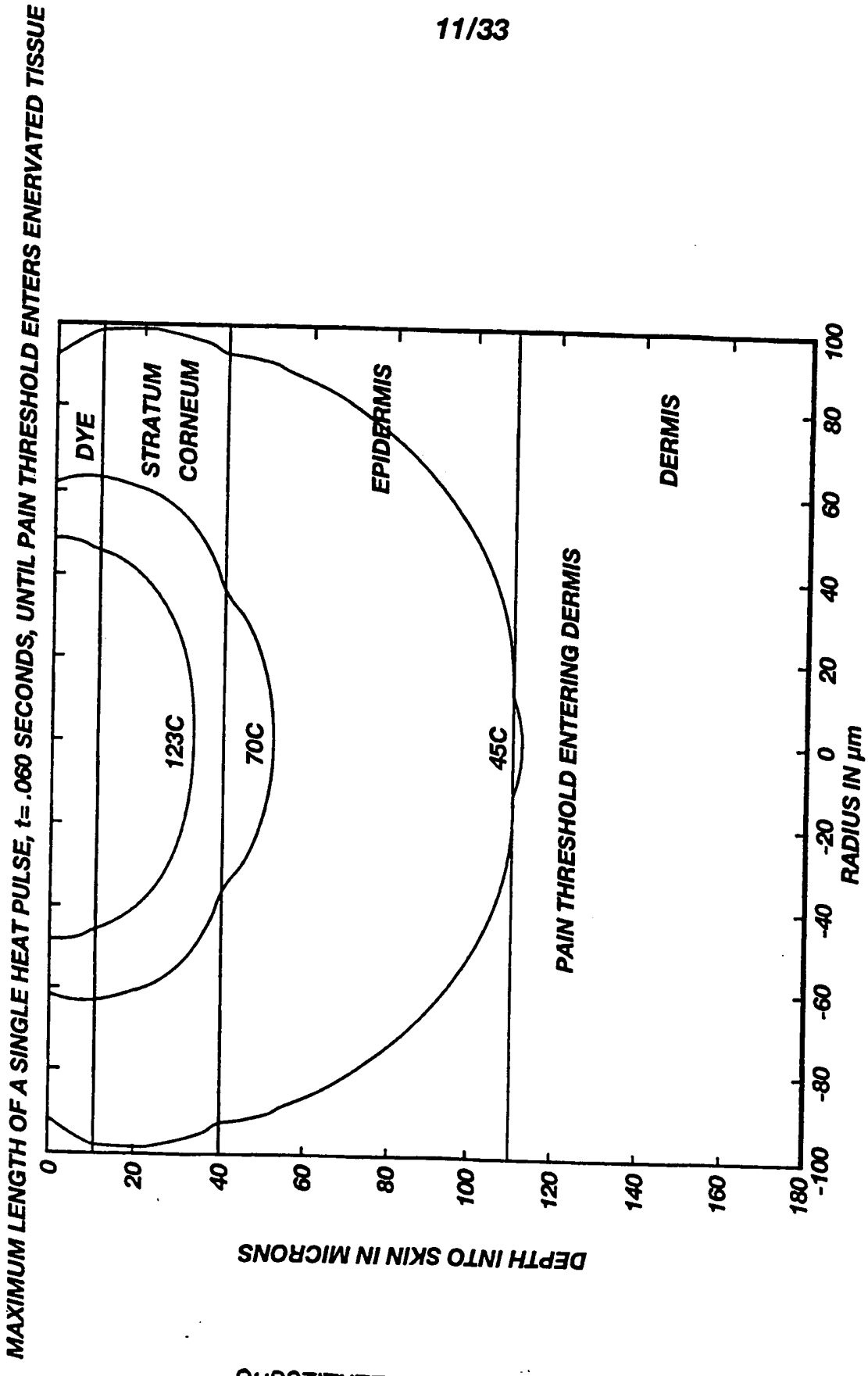
MAXIMUM LENGTH OF A SINGLE HEAT PULSE, t= .060 SECONDS, UNTIL PAIN THRESHOLD ENTERS ENERVATED TISSUE
DYE
STRATUM CORNEUM
EPIDERMIS
DERMIS
123C
70C
45C
PAIN THRESHOLD ENTERING DERMIS
0
20
40
60
80
100
120
140
160
180
DEPTH INTO SKIN IN MICRONS
-100
-80
-60
-40
-20
0
20
40
60
80
100
RADIUS IN µm

Fig. 10

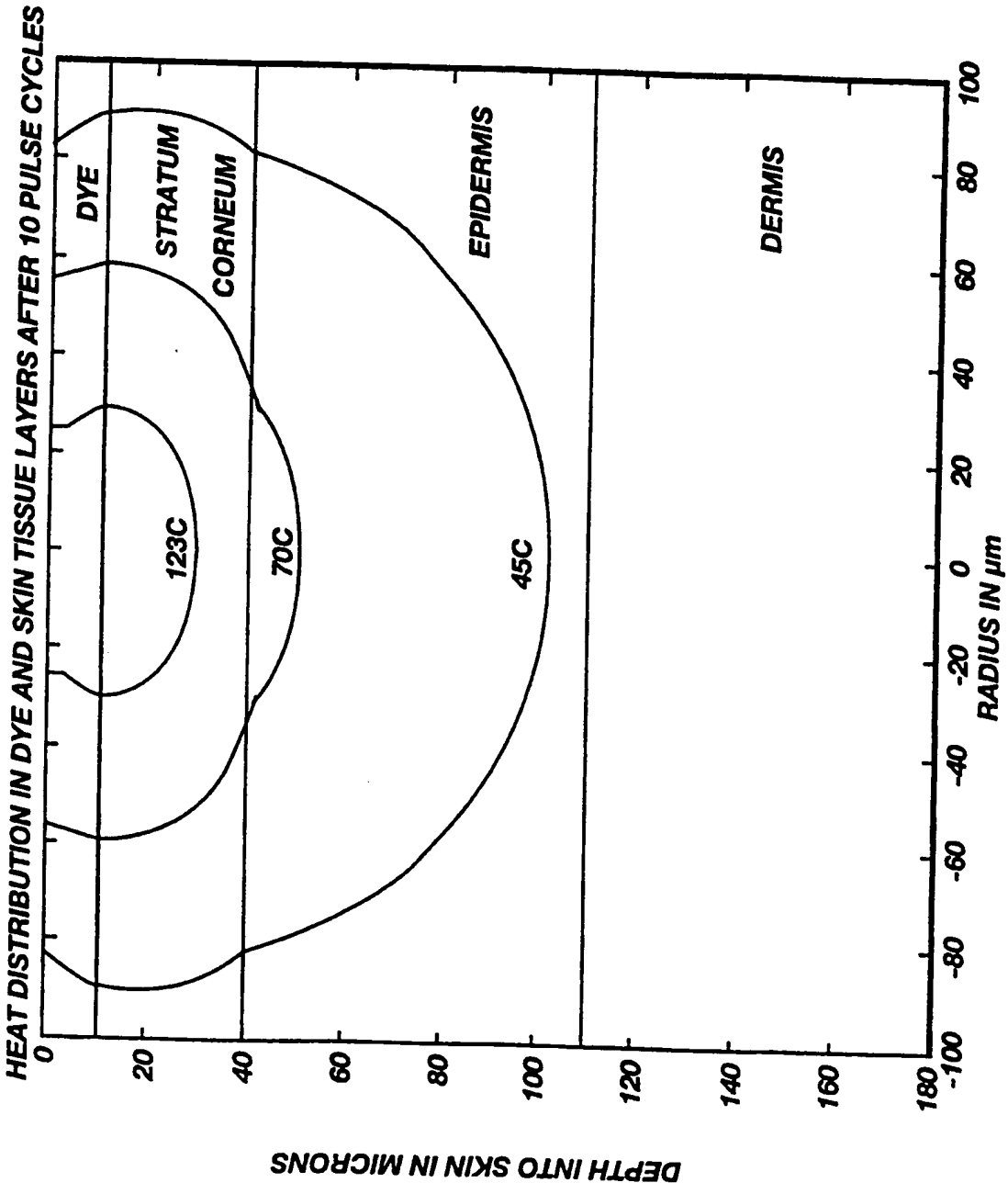
HEAT DISTRIBUTION IN DYE AND SKIN TISSUE LAYERS AFTER 10 PULSE CYCLES
DYE
STRATUM CORNEUM
EPIDERMIS
DERMIS
123C
70C
45C
0
20
40
60
80
100
120
140
160
180
-100
-80
-60
-40
-20
0
20
40
60
80
100
RADIUS IN µm
DEPTH INTO SKIN IN MICRONS

Fig. 11

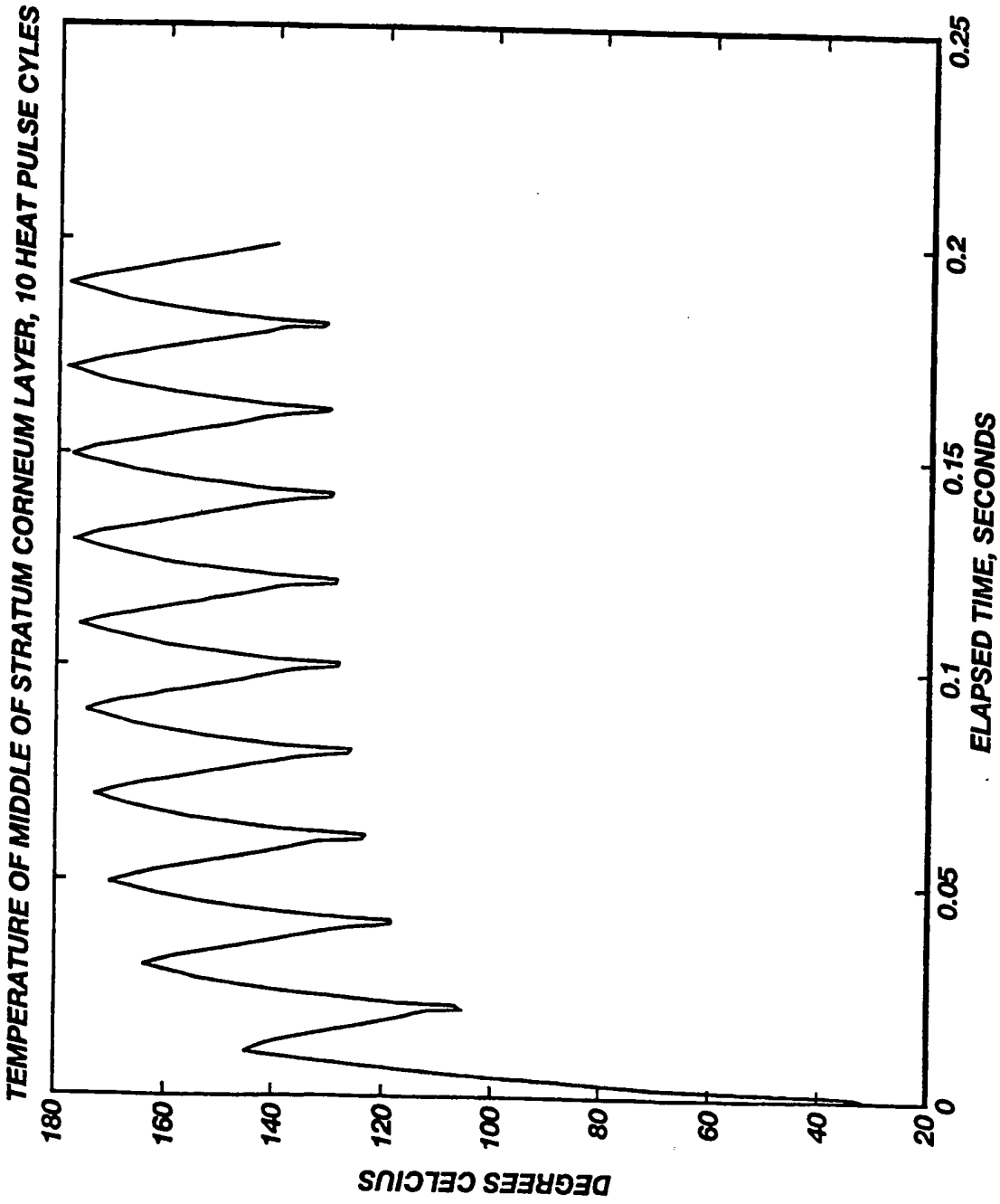
TEMPERATURE OF MIDDLE OF STRATUM CORNEUM LAYER, 10 HEAT PULSE CYLES
DEGREES CELCIUS
180
160
140
120
100
80
60
40
20
0
0.05
0.1
0.15
0.2
0.25
ELAPSED TIME, SECONDS

Fig. 12

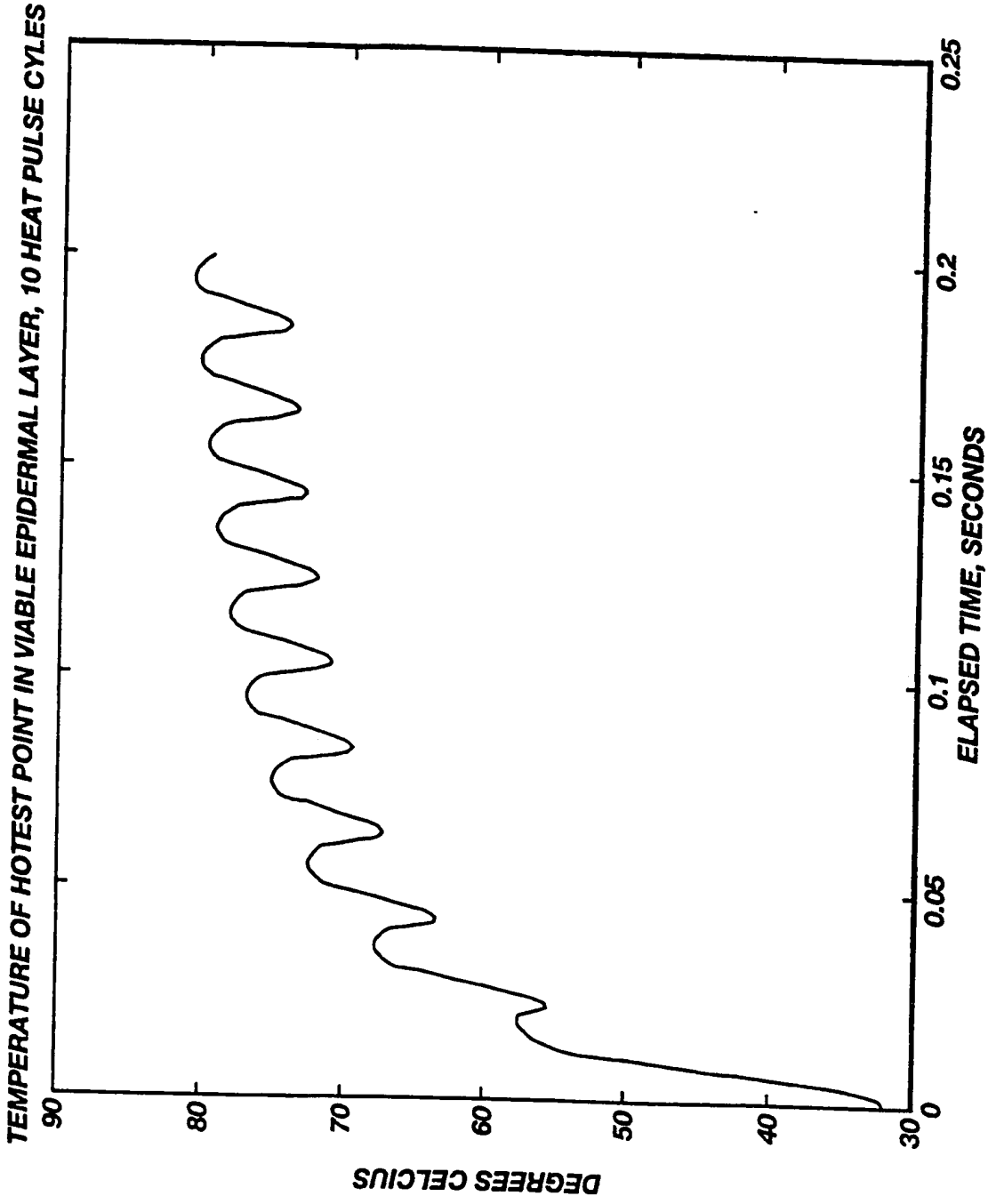
TEMPERATURE OF HOTEST POINT IN VIABLE EPIDERMAL LAYER, 10 HEAT PULSE CYLES
DEGREES CELCIUS
90
80
70
60
50
40
30
0
0.05
0.1
0.15
0.2
0.25
ELAPSED TIME, SECONDS

Fig. 13

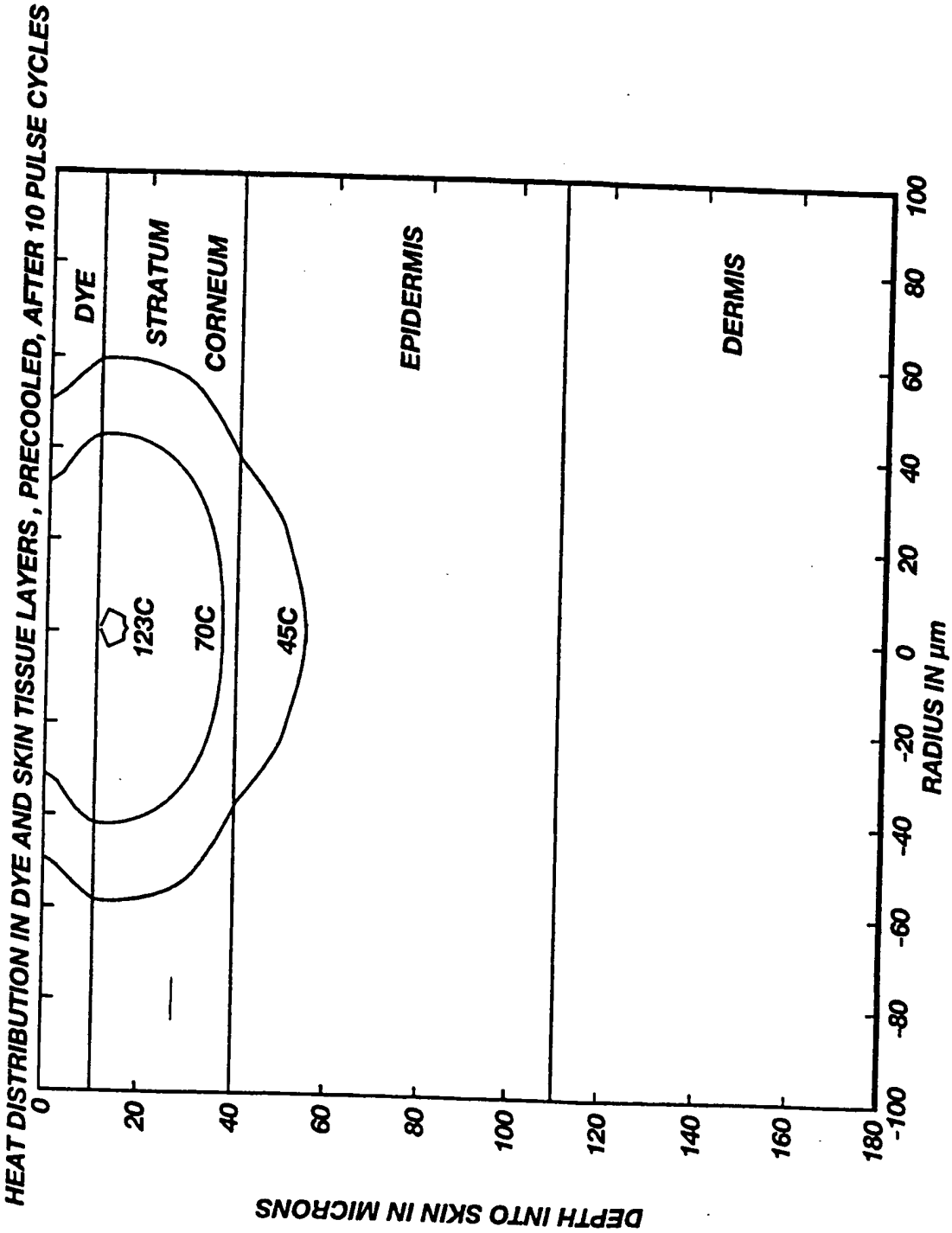
HEAT DISTRIBUTION IN DYE AND SKIN TISSUE LAYERS, PRECOOLED, AFTER 10 PULSE CYCLES
DYE
STRATUM CORNEUM
EPIDERMIS
DERMIS
123C
70C
45C
0
20
40
60
80
100
120
140
160
180
-100
-80
-60
-40
-20
0
20
40
60
80
100
RADIUS IN µm
DEPTH INTO SKIN IN MICRONS

Fig. 14

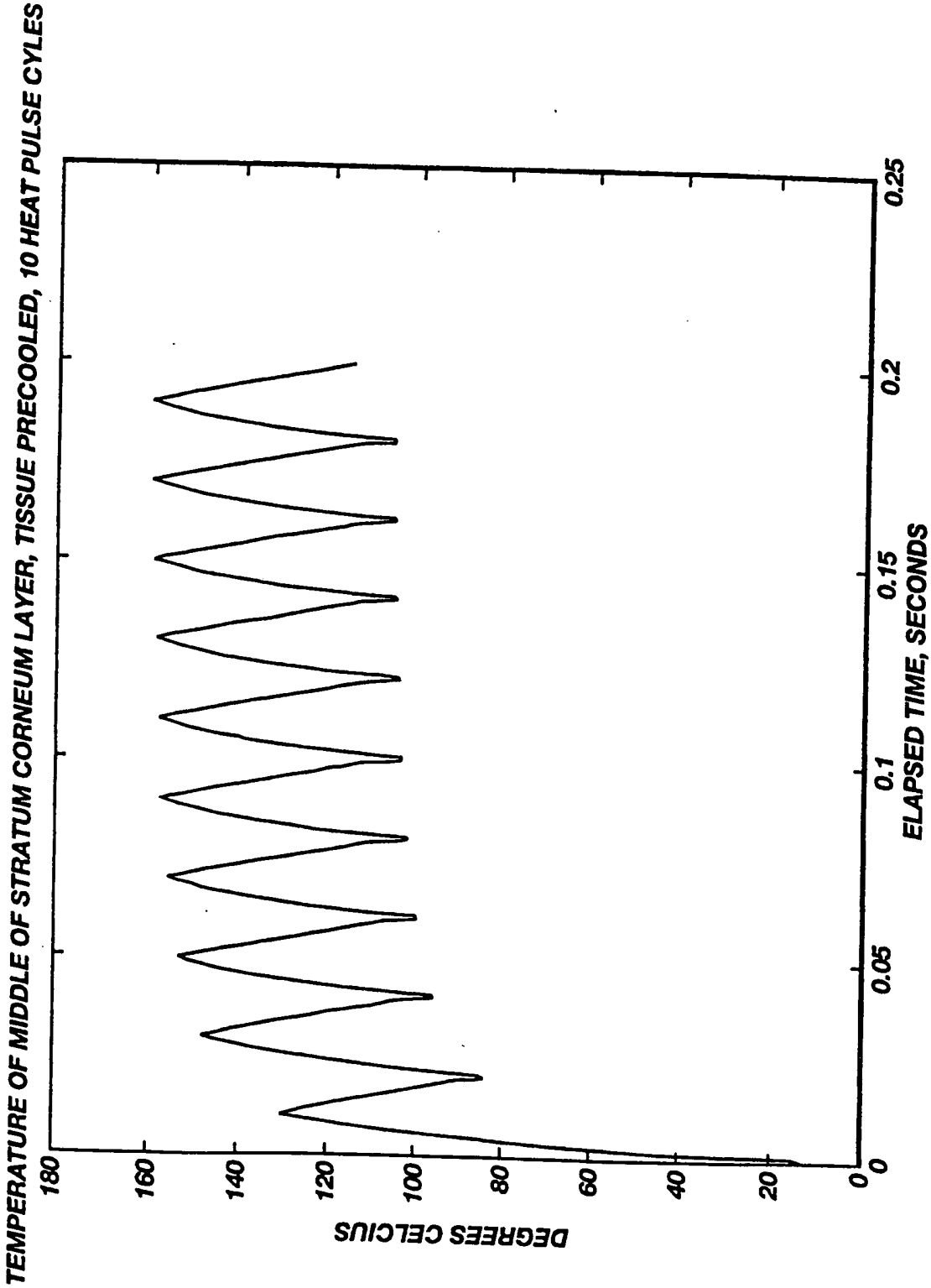
TEMPERATURE OF MIDDLE OF STRATUM CORNEUM LAYER, TISSUE PRECOOLED, 10 HEAT PULSE CYLES
DEGREES CELCIUS
180
160
140
120
100
80
60
40
20
0
0
0.05
0.1
0.15
0.2
0.25
ELAPSED TIME, SECONDS

Fig. 15

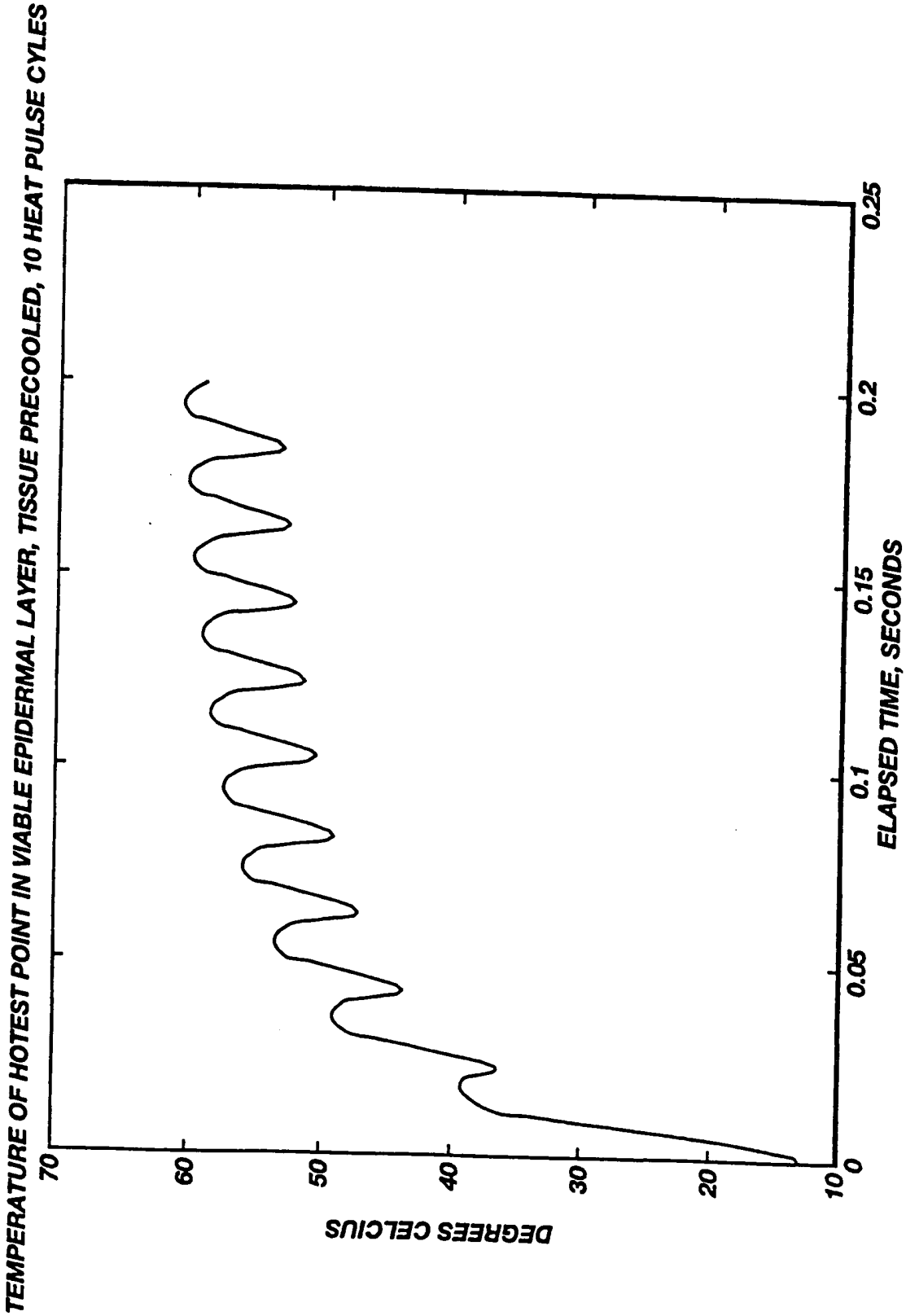
TEMPERATURE OF HOTEST POINT IN VIABLE EPIDERMAL LAYER, TISSUE PRECOOLED, 10 HEAT PULSE CYLES
DEGREES CELCIUS
70
60
50
40
30
20
10
0
0.05
0.1
0.15
0.2
0.25
ELAPSED TIME, SECONDS

Fig. 16

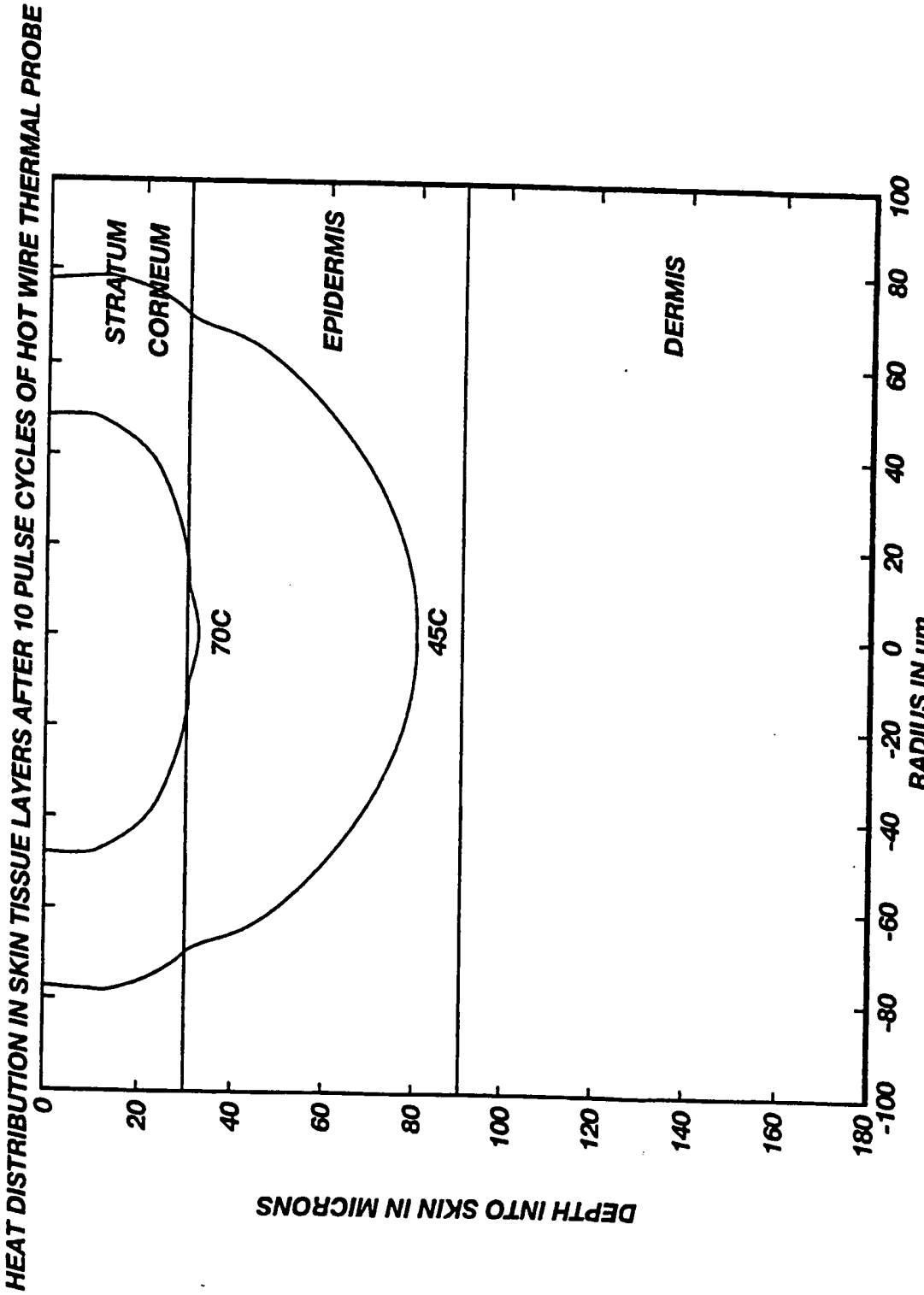
HEAT DISTRIBUTION IN SKIN TISSUE LAYERS AFTER 10 PULSE CYCLES OF HOT WIRE THERMAL PROBE
STRATUM CORNEUM
EPIDERMIS
DERMIS
70C
45C
0
20
40
60
80
100
120
140
160
180
DEPTH INTO SKIN IN MICRONS
100
80
60
40
20
0
-20
-40
-60
-80
-100
RADIUS IN µm

Fig. 17

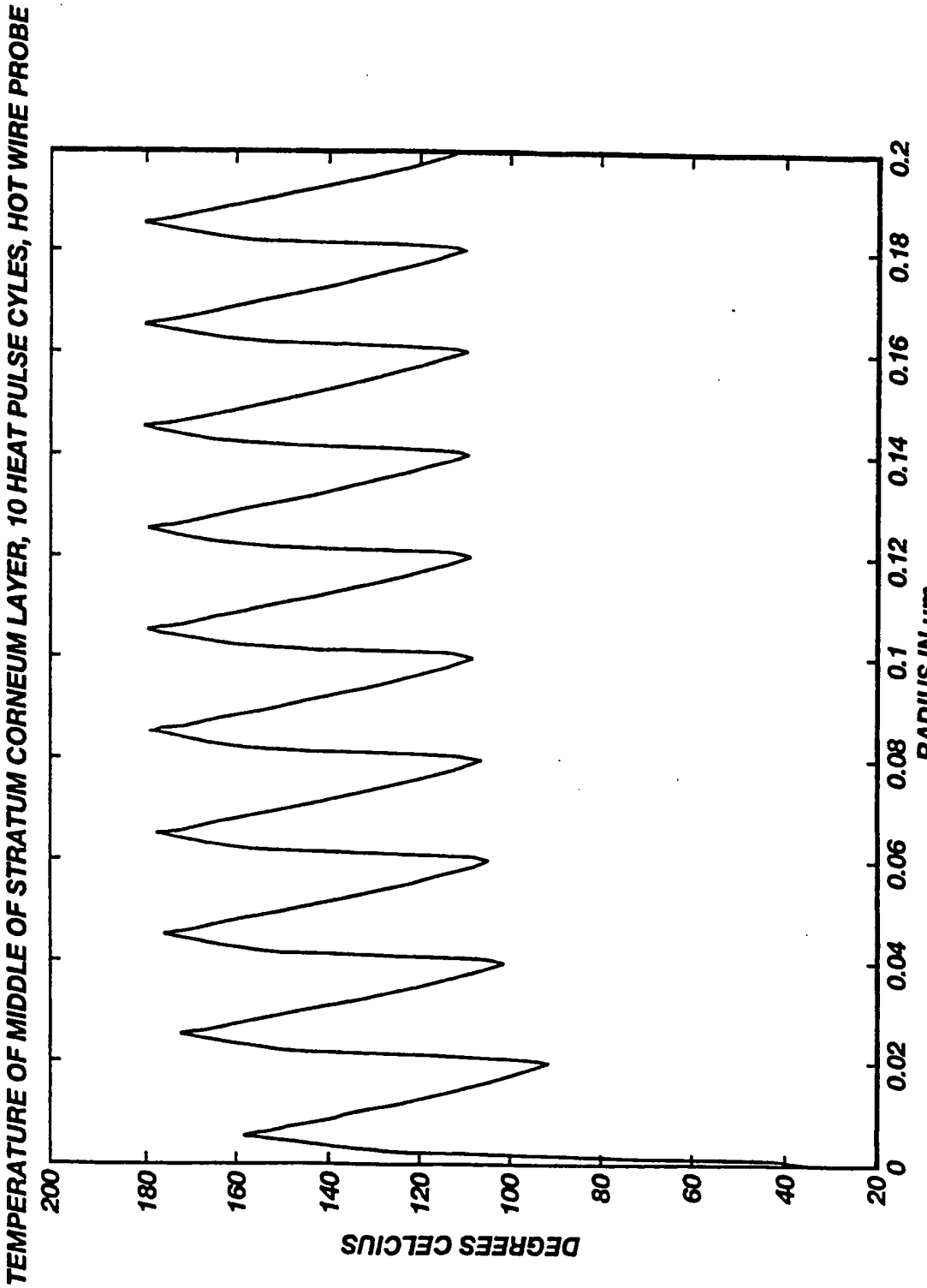
TEMPERATURE OF MIDDLE OF STRATUM CORNEUM LAYER, 10 HEAT PULSE CYLES, HOT WIRE PROBE
DEGREES CELCIUS
200
180
160
140
120
100
80
60
40
20
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
RADIUS IN µm

Fig. 18

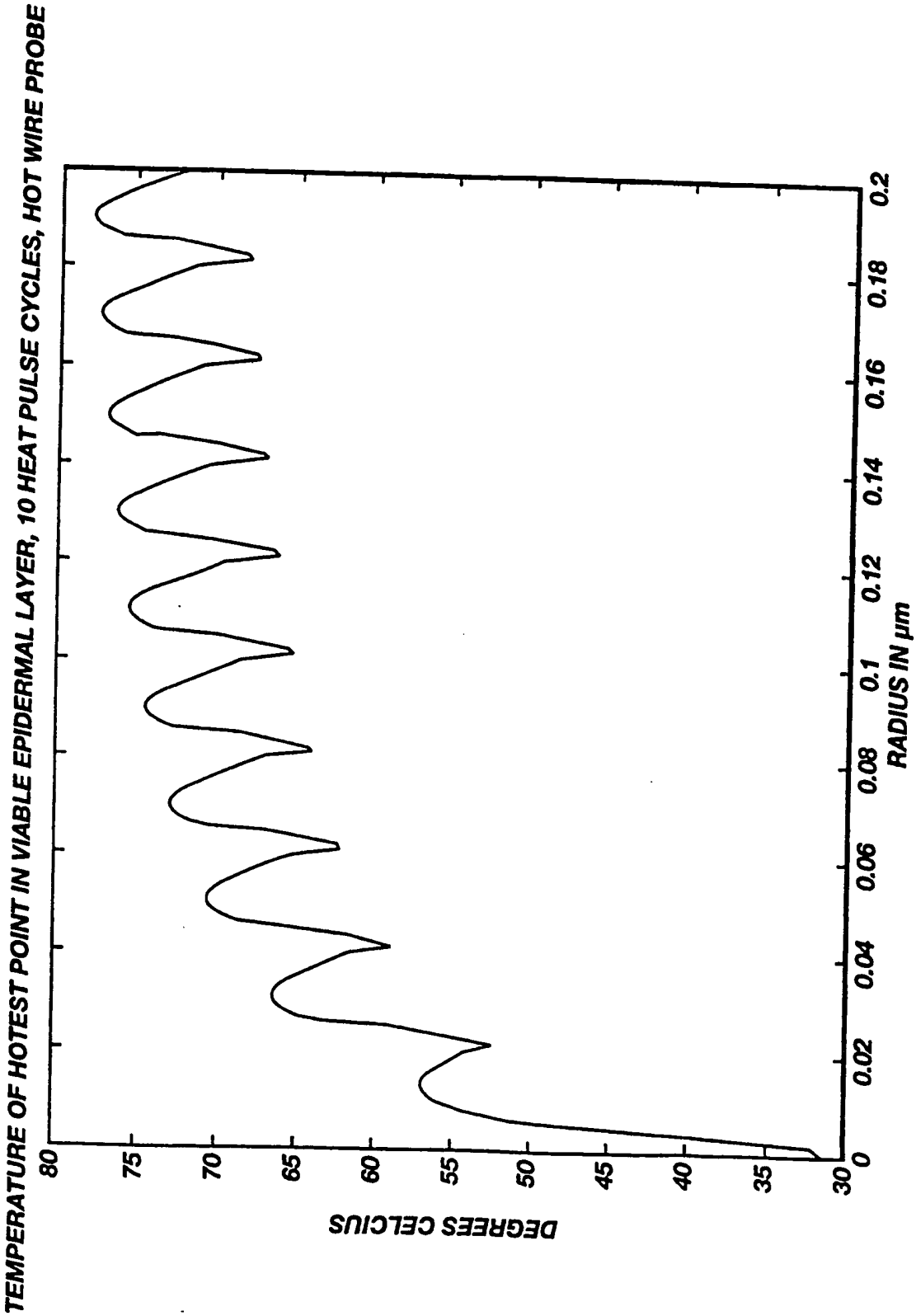
TEMPERATURE OF HOTEST POINT IN VIABLE EPIDERMAL LAYER, 10 HEAT PULSE CYCLES, HOT WIRE PROBE
DEGREES CELCIUS
80
75
70
65
60
55
50
45
40
35
30
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
RADIUS IN µm

Fig. 19

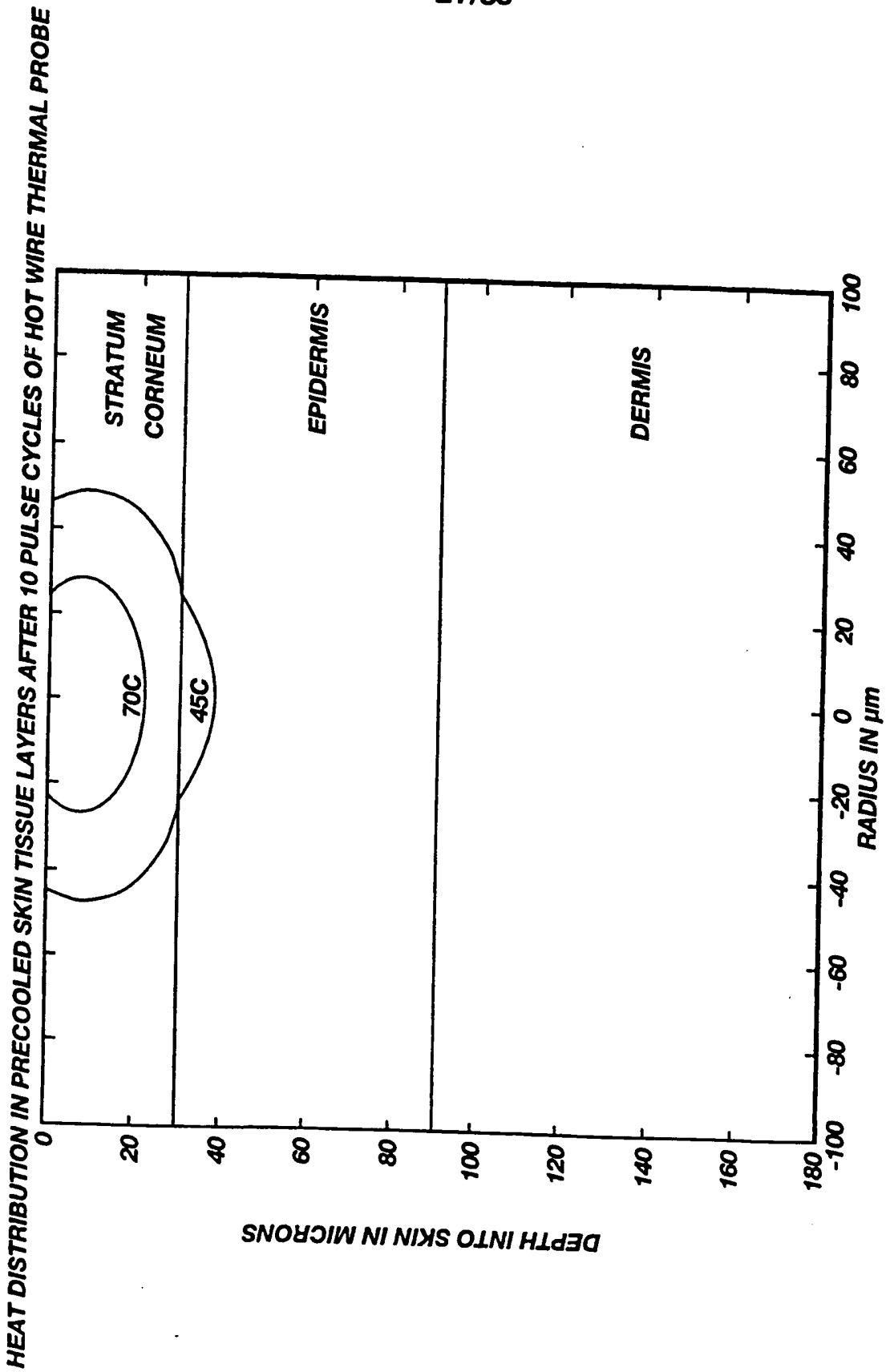
HEAT DISTRIBUTION IN PRECOOLED SKIN TISSUE LAYERS AFTER 10 PULSE CYCLES OF HOT WIRE THERMAL PROBE
STRATUM CORNEUM
EPIDERMIS
DERMIS
70C
45C
0
20
40
60
80
100
120
140
160
180
DEPTH INTO SKIN IN MICRONS
100
80
60
40
20
0
-20
-40
-60
-80
-100
RADIUS IN µm

Fig. 20

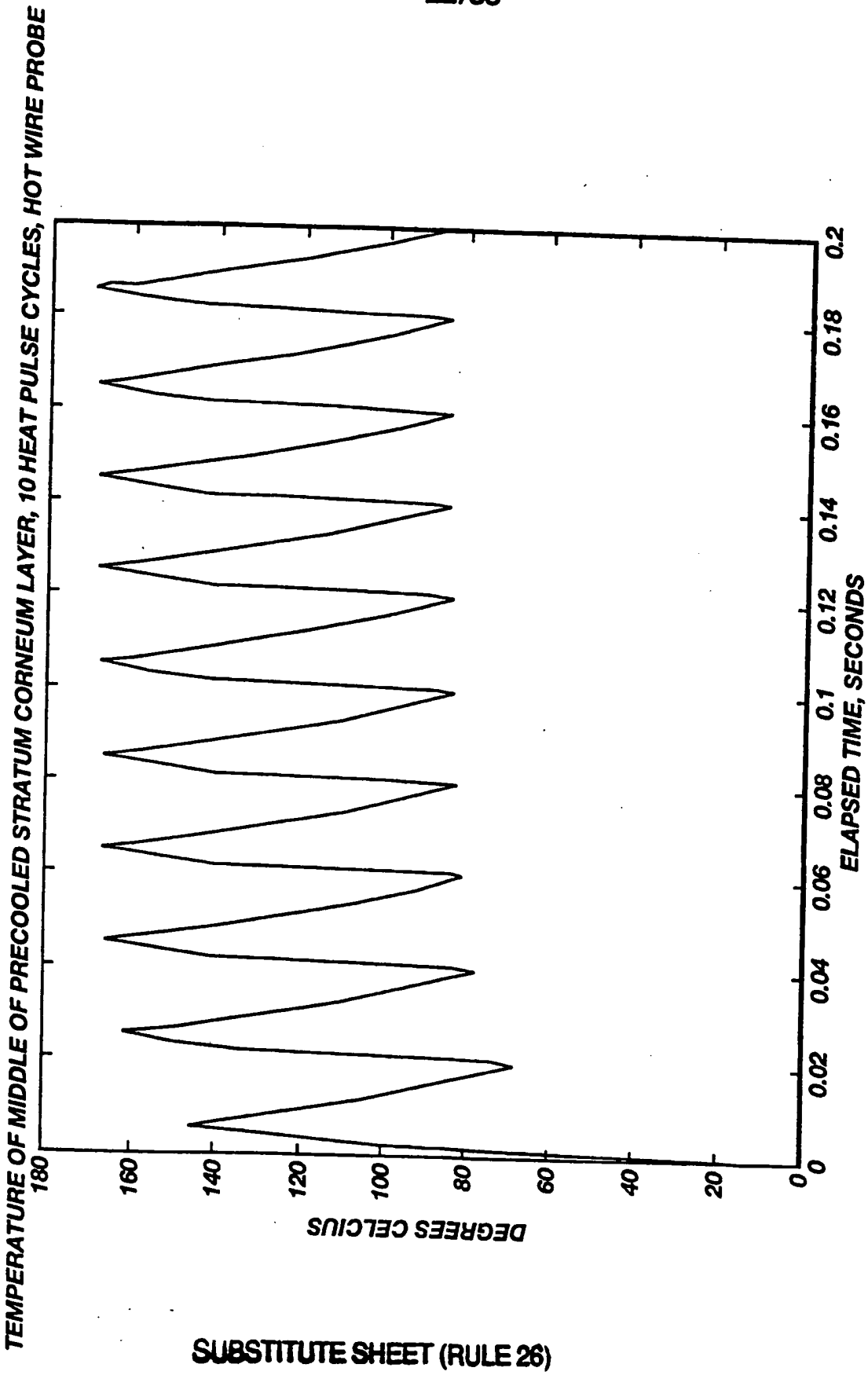
TEMPERATURE OF MIDDLE OF PRECOOLED STRATUM CORNEUM LAYER, 10 HEAT PULSE CYCLES, HOT WIRE PROBE
DEGREES CELCIUS
0
20
40
60
80
100
120
140
160
180
ELAPSED TIME, SECONDS
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2

Fig. 21

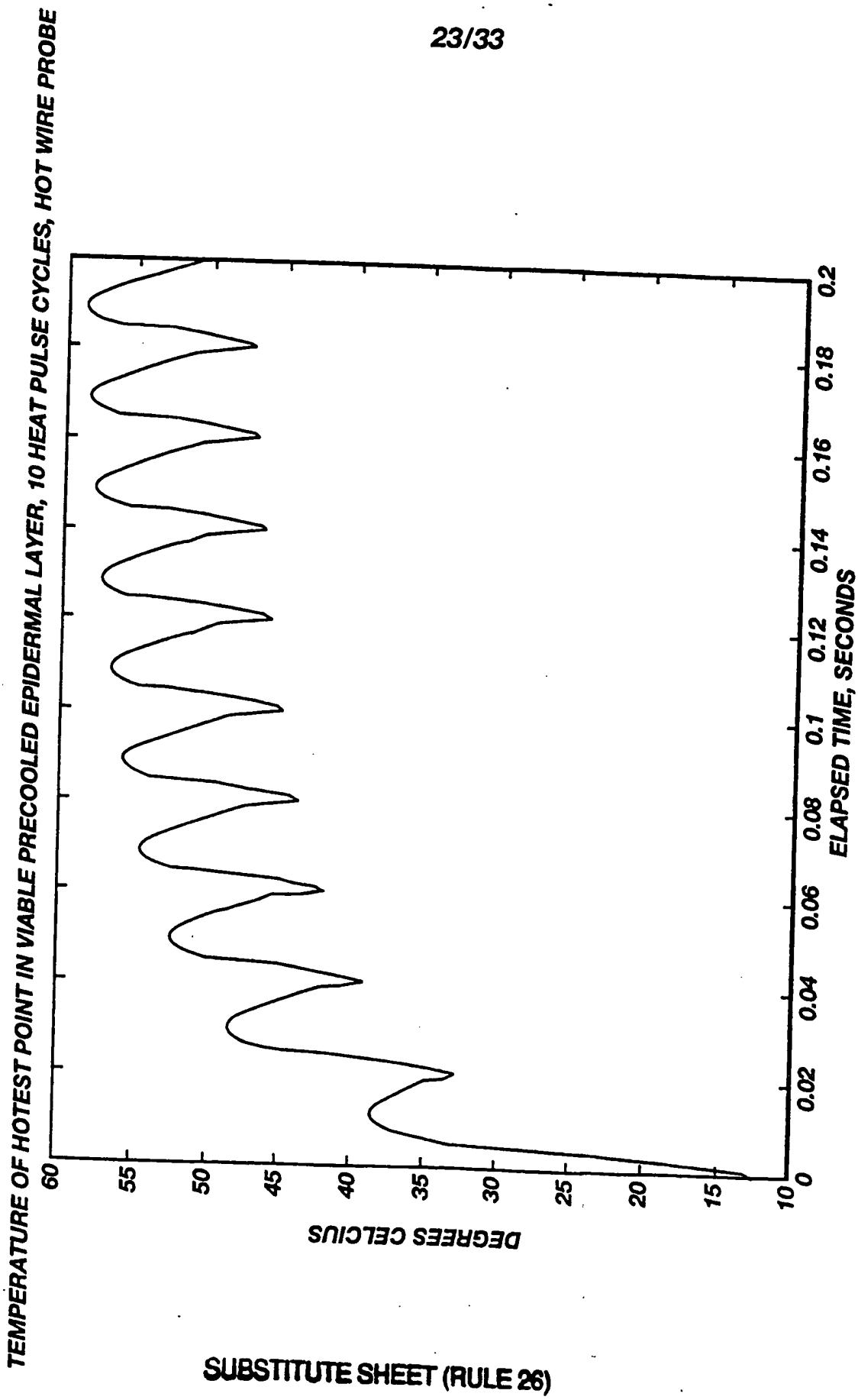
TEMPERATURE OF HOTEST POINT IN VIABLE PRECOOLED EPIDERMAL LAYER, 10 HEAT PULSE CYCLES, HOT WIRE PROBE
DEGREES CELCIUS
60
55
50
45
40
35
30
25
20
15
10
0
0.02
0.04
0.06
0.08
0.1
0.12
0.14
0.16
0.18
0.2
ELAPSED TIME, SECONDS

Fig. 22

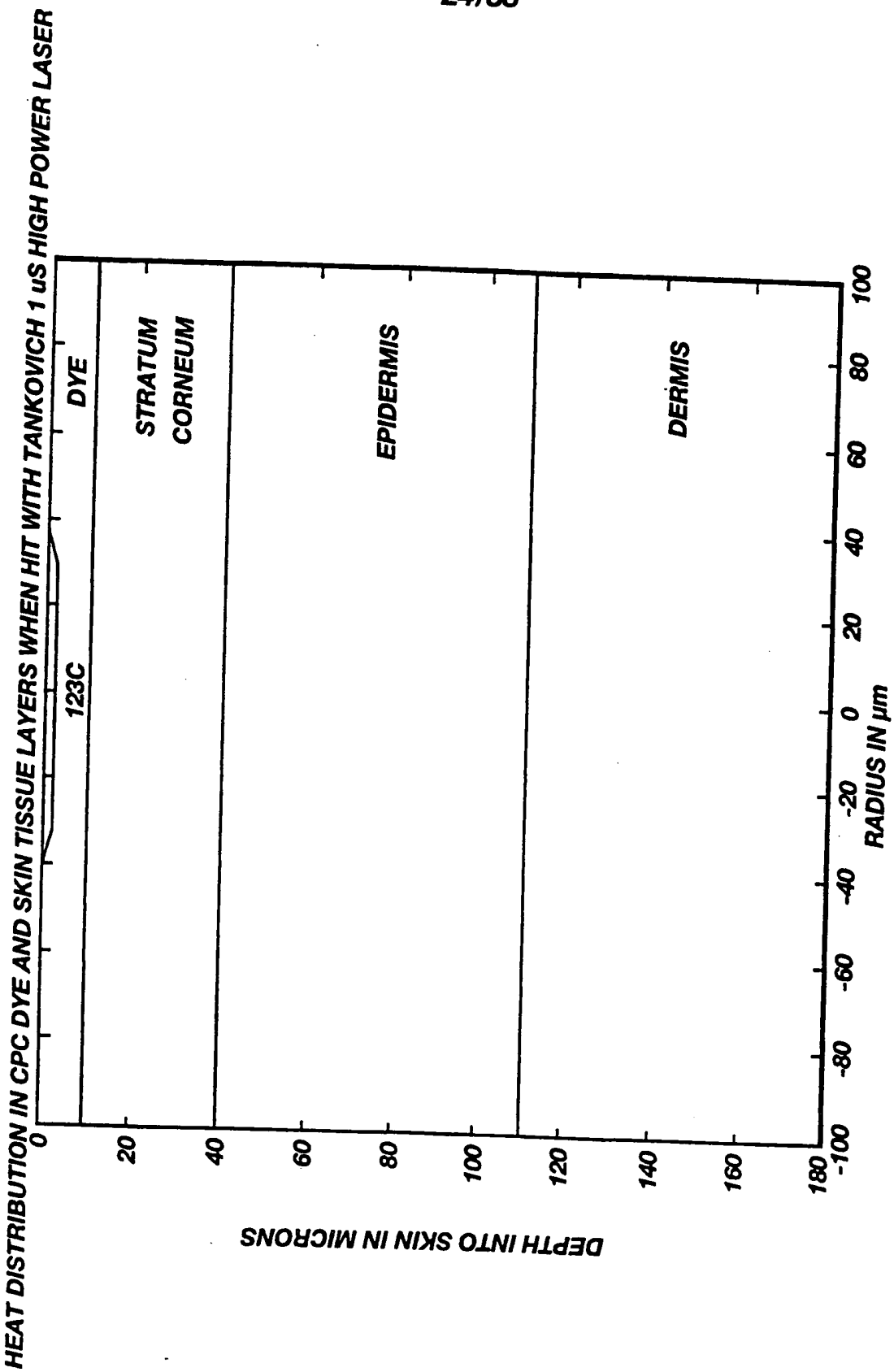
HEAT DISTRIBUTION IN CPC DYE AND SKIN TISSUE LAYERS WHEN HIT WITH TANKOVICH 1 uS HIGH POWER LASER
DYE
123C
STRATUM CORNEUM
EPIDERMIS
DERMIS
0
20
40
60
80
100
120
140
160
180
DEPTH INTO SKIN IN MICRONS
100
80
60
40
20
0
-20
-40
-60
-80
-100
RADIUS IN µm

Fig. 23

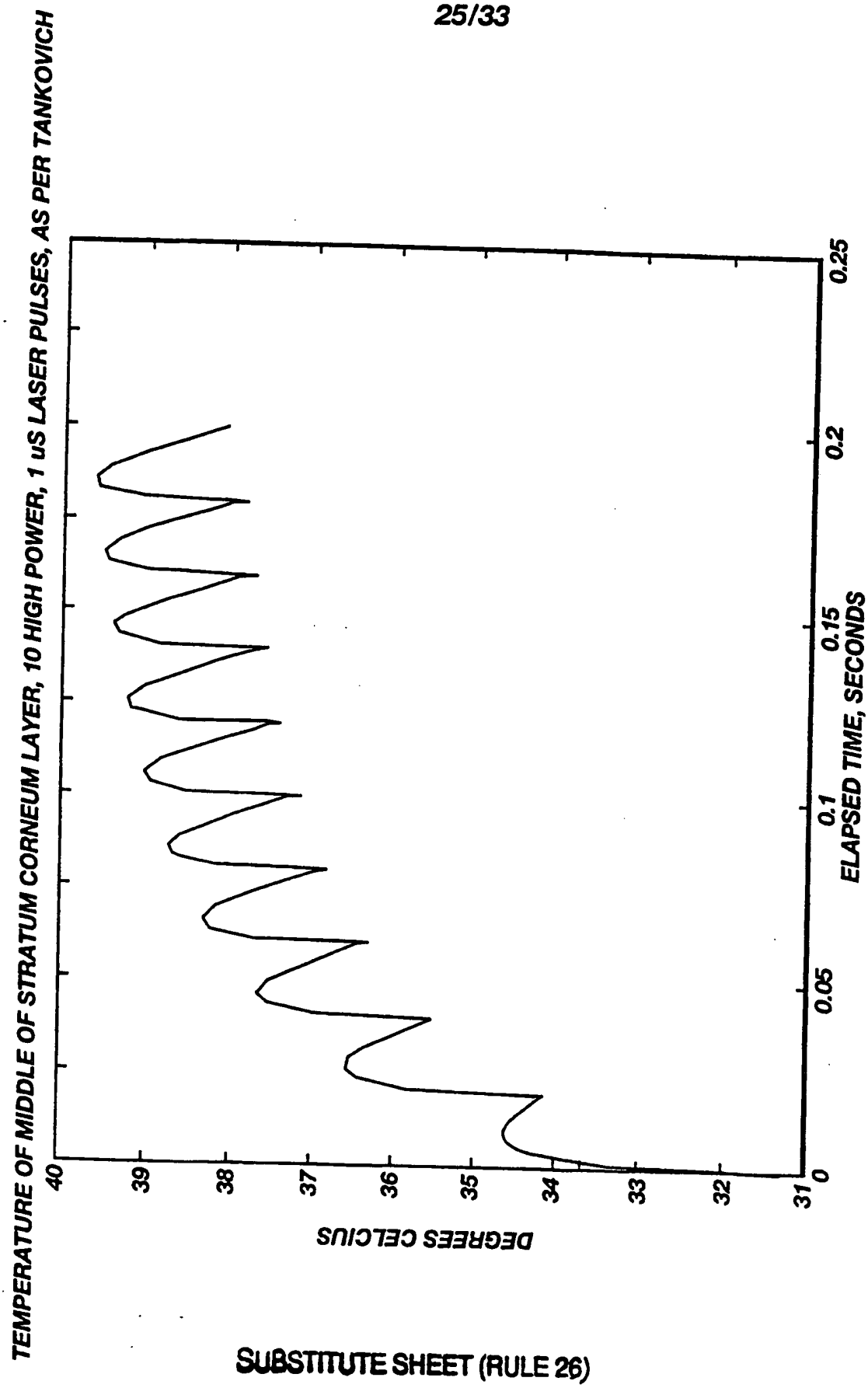
TEMPERATURE OF MIDDLE OF STRATUM CORNEUM LAYER, 10 HIGH POWER, 1 uS LASER PULSES, AS PER TANKOVICH
DEGREES CELCIUS
40
39
38
37
36
35
34
33
32
31
0
0.05
0.1
0.15
0.2
0.25
ELAPSED TIME, SECONDS

Fig. 24

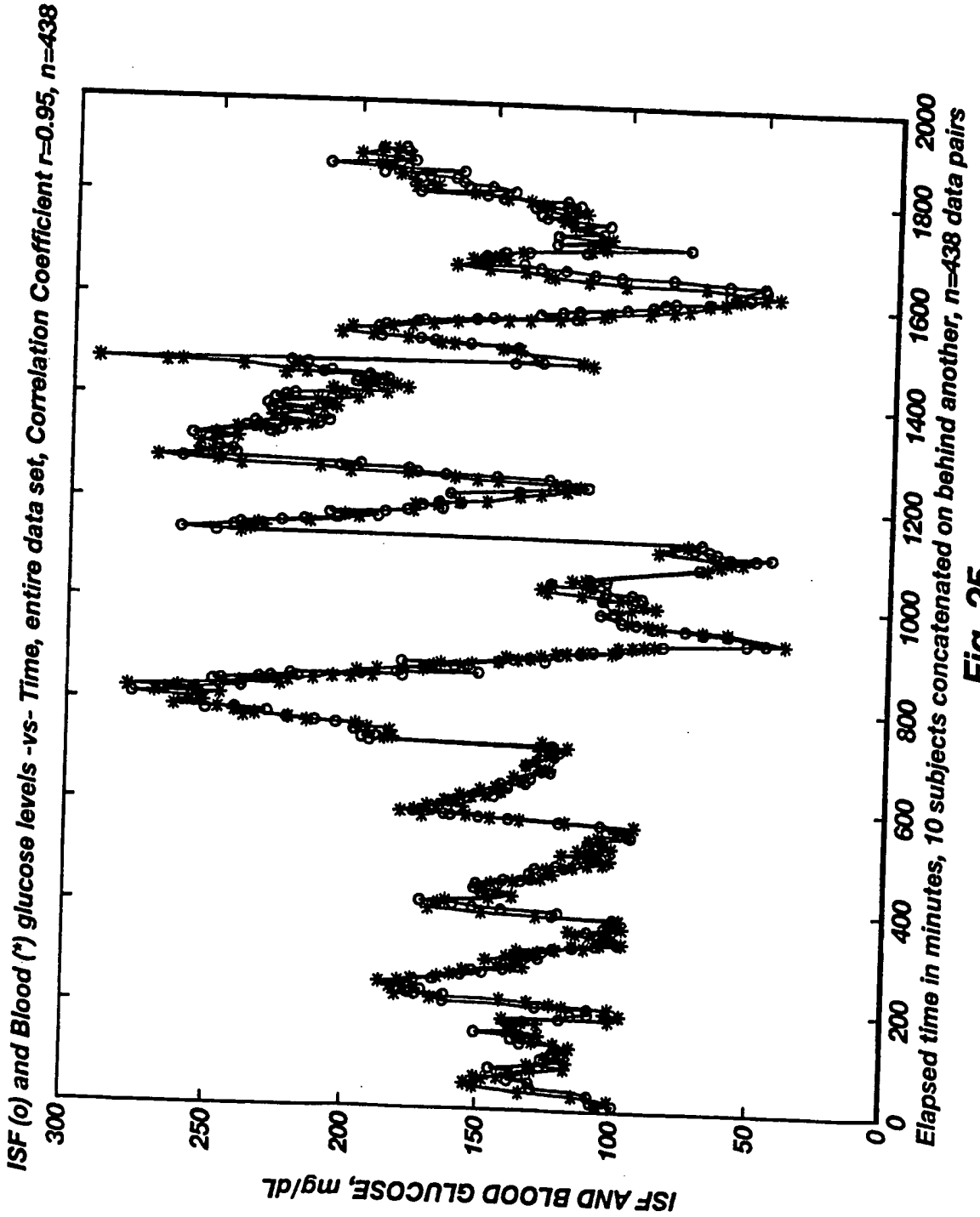
ISF (o) and Blood (*) glucose levels -vs- Time, entire data set, Correlation Coefficient r=0.95, n=438
ISF AND BLOOD GLUCOSE, mg/dL
300
250
200
150
100
50
0
0
200
400
600
800
1000
1200
1400
1600
1800
2000
Elapsed time in minutes, 10 subjects concatenated on behind another, n=438 data pairs

Fig. 25

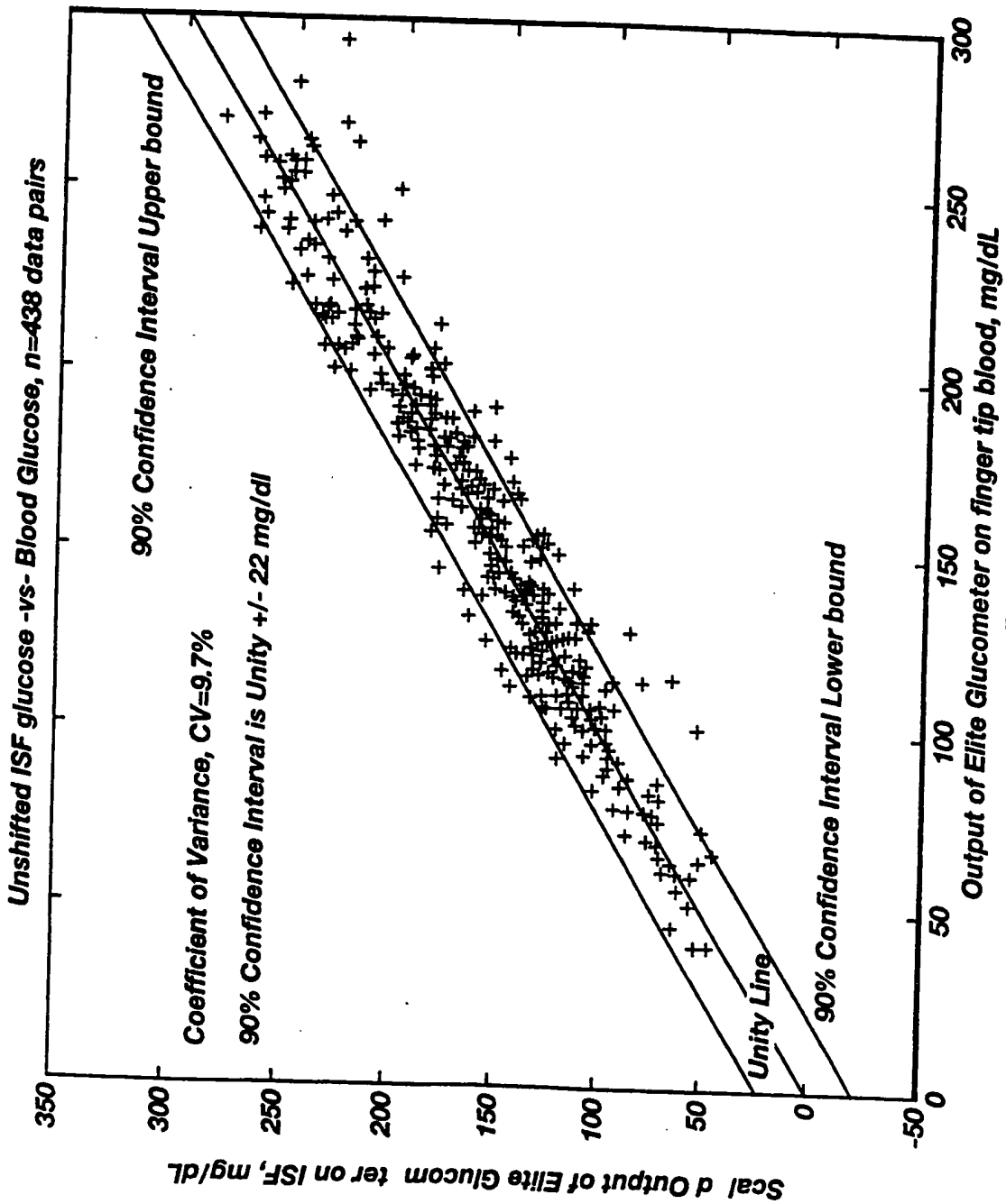
Unshifted ISF glucose -vs- Blood Glucose, n=438 data pairs
90% Confidence Interval Upper bound
Coefficient of Variance, CV=9.7%
90% Confidence Interval is Unity +/- 22 mg/dl
Unity Line
90% Confidence Interval Lower bound
Scal d Output of Elite Glucom ter on ISF, mg/dL
Output of Elite Glucometer on finger tip blood, mg/dL
350
300
250
200
150
100
50
0
-50
0
50
100
150
200
250
300

Fig. 26

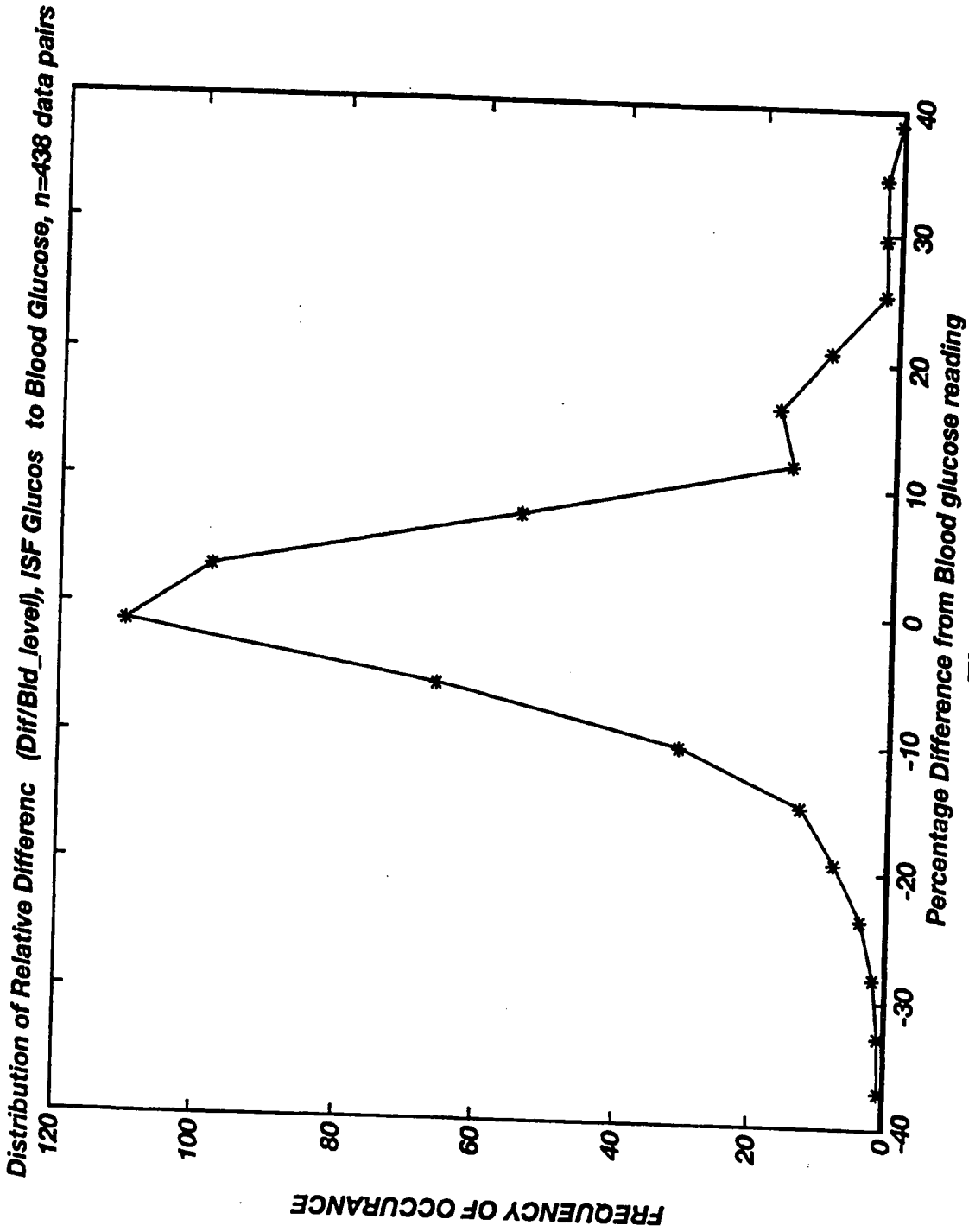
Distribution of Relative Differenc (Dif/Bld_level), ISF Glucos to Blood Glucose, n=438 data pairs
FREQUENCY OF OCCURANCE
120
100
80
60
40
20
0
-40
-30
-20
-10
0
10
20
30
40
Percentage Difference from Blood glucose reading

Fig. 27

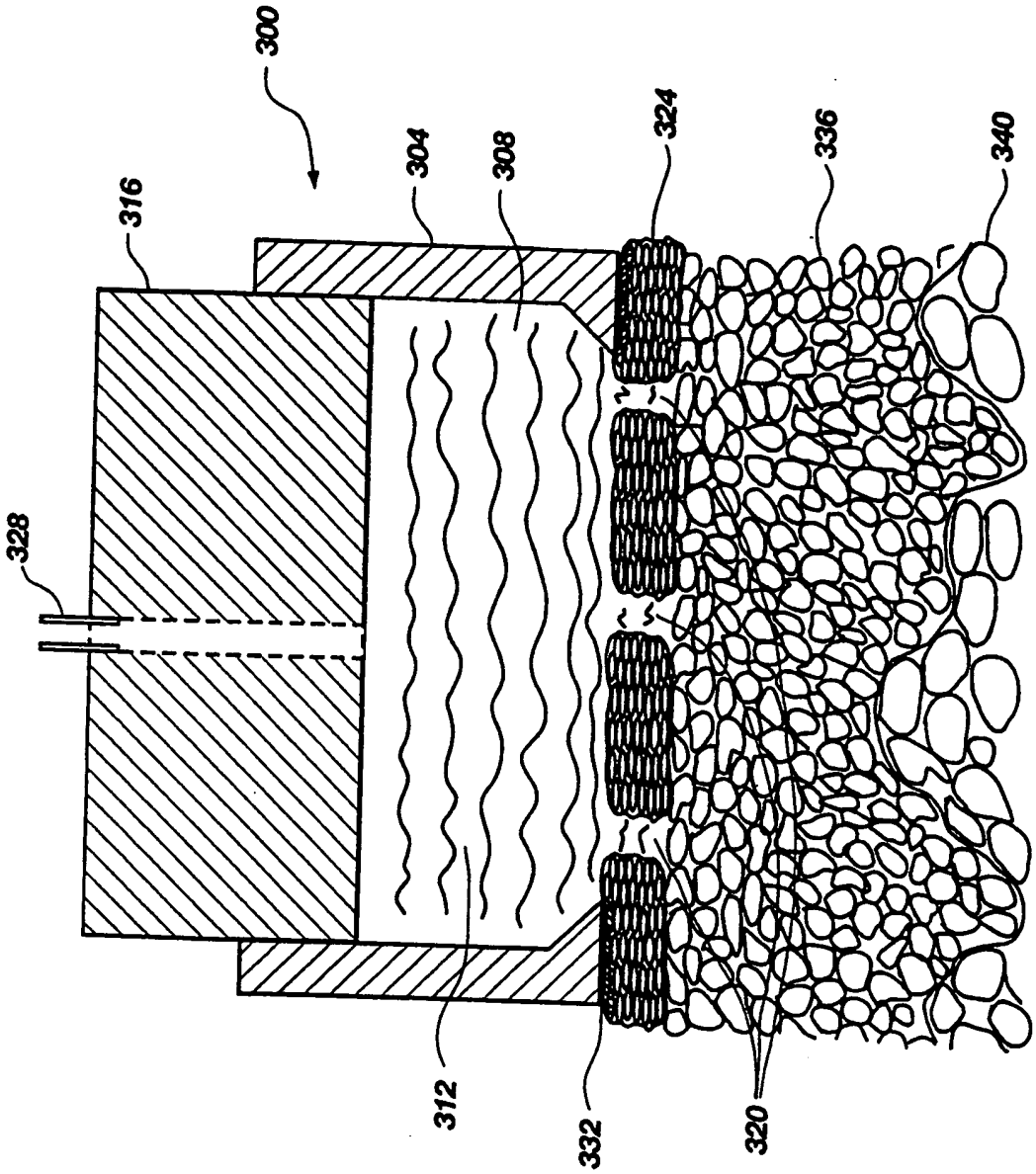

Fig. 28

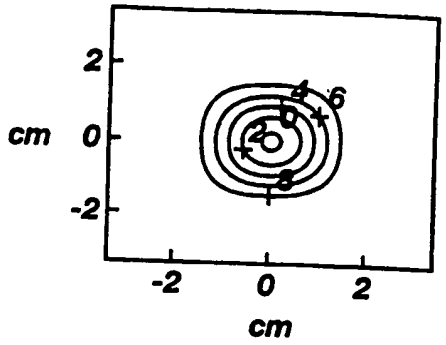

Fig. 29A

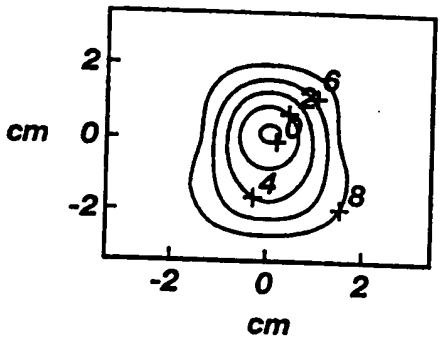

Fig. 29B

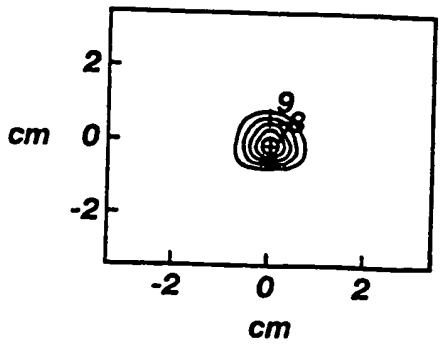

Fig. 29C

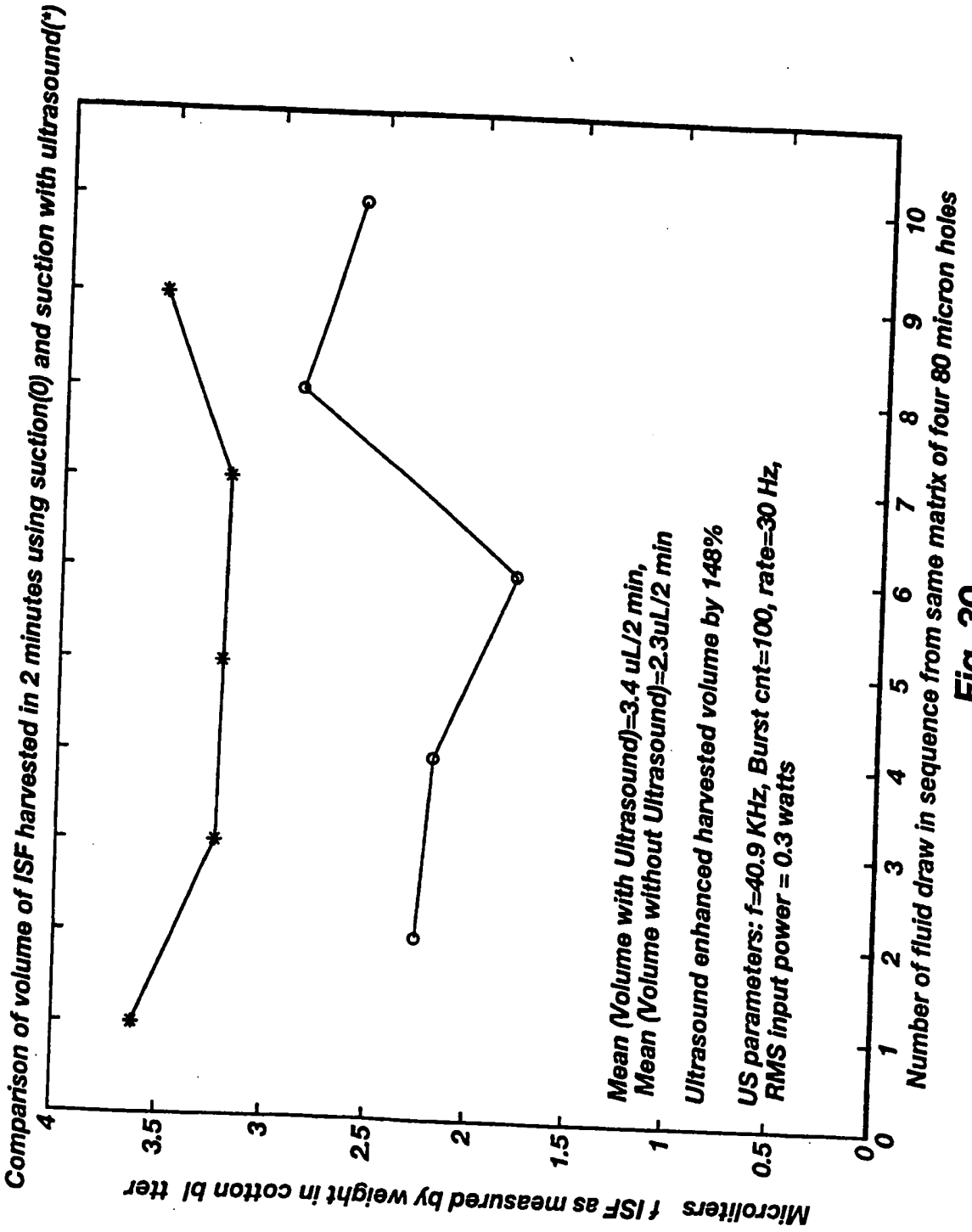
Comparison of volume of ISF harvested in 2 minutes using suction(0) and suction with ultrasound(*)
Microliters of ISF as measured by weight in cotton blotter
Number of fluid draw in sequence from same matrix of four 80 micron holes
Mean (Volume with Ultrasound)=3.4 uL/2 min,
Mean (Volume without Ultrasound)=2.3uL/2 min
Ultrasound enhanced harvested volume by 148%
US parameters: f=40.9 KHz, Burst cnt=100, rate=30 Hz,
RMS input power = 0.3 watts
0
0.5
1
1.5
2
2.5
3
3.5
4
1
2
3
4
5
6
7
8
9
10

Fig. 30

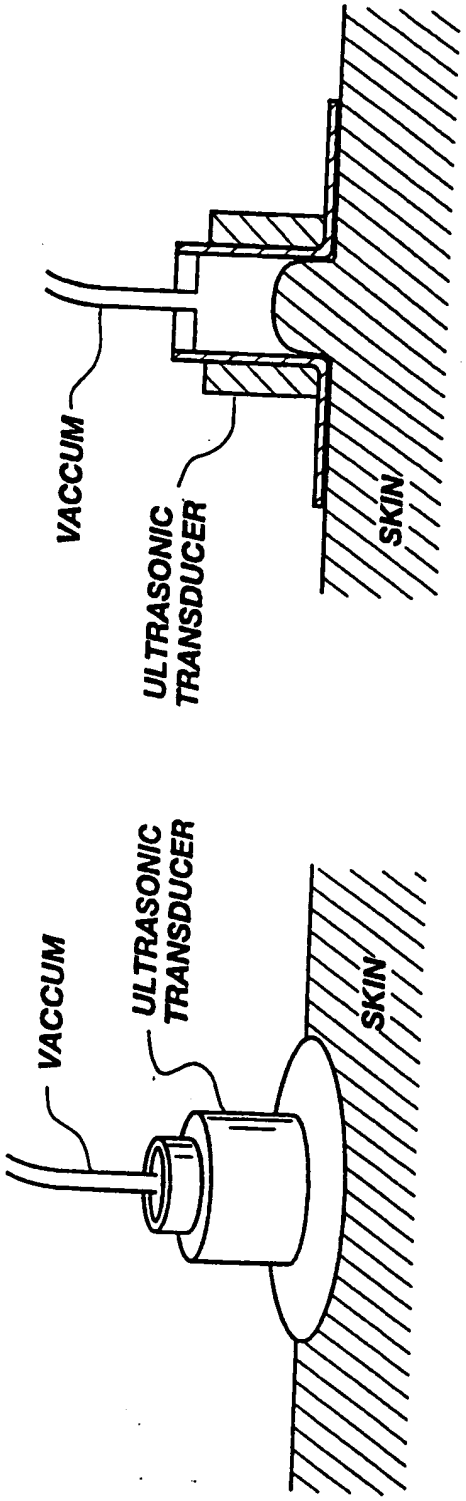

Fig. 31

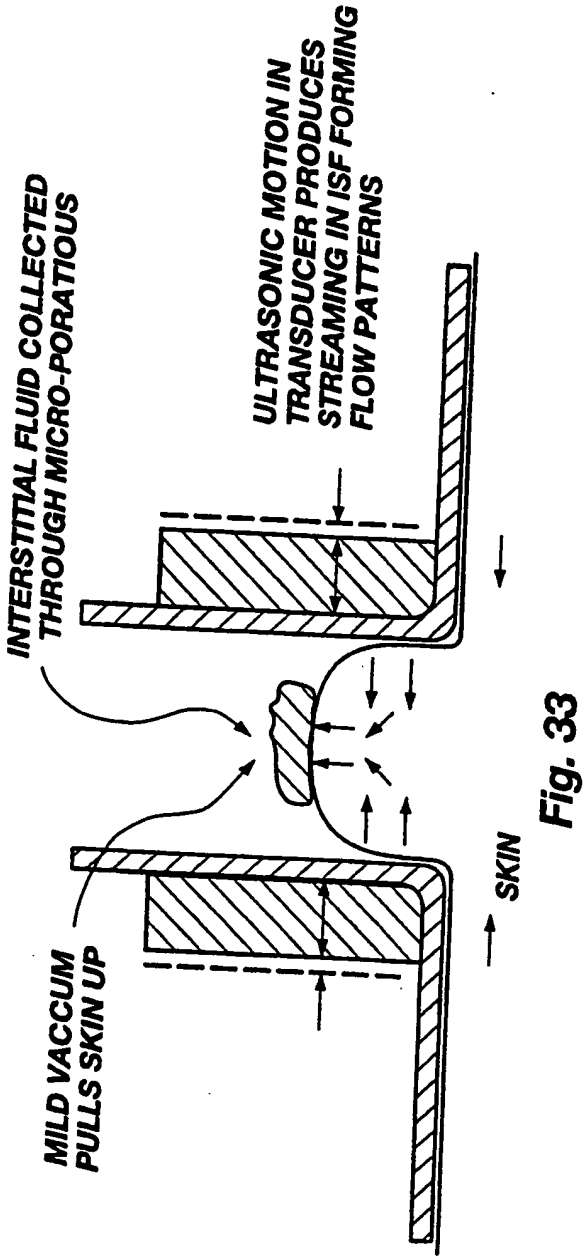

Fig. 32

Fig. 33

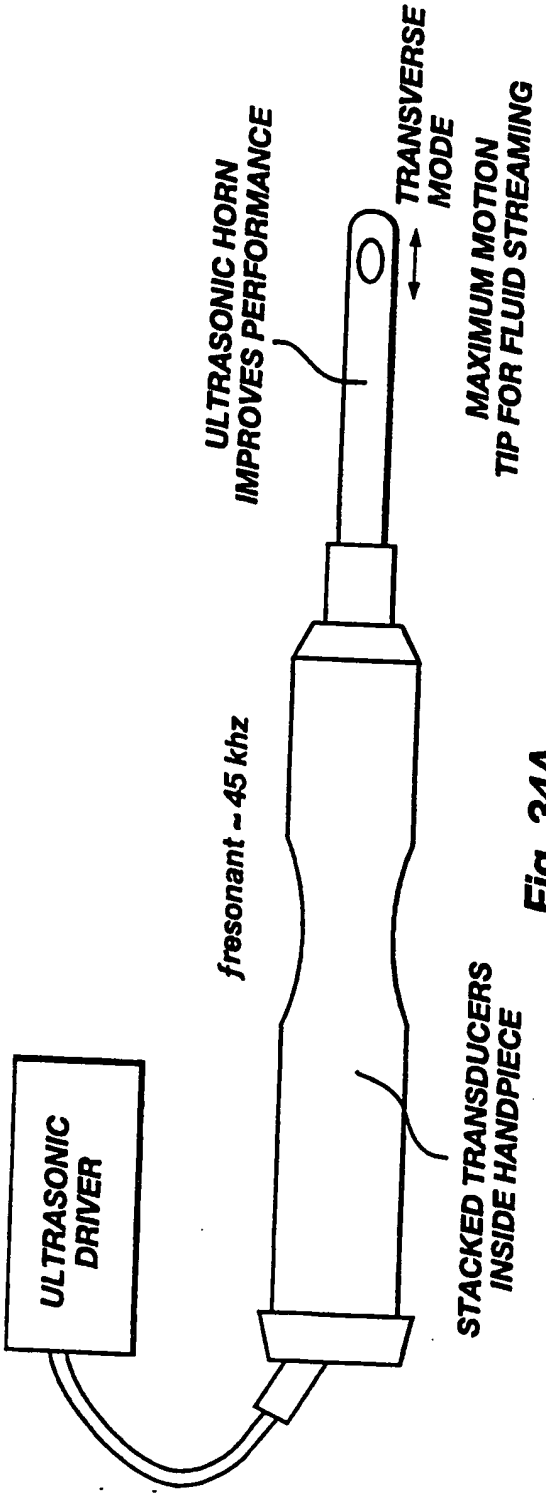

Fig. 34A

Fig. 34B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/13865

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61/B 5/00; A61M 37/00
US CL :128/632, 633, 760, 771; 604/49, 290
According to International Patent Classification (IPC) r to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 128/632, 633, 636, 637, 760, 771; 601/2; 604/20, 22, 23, 289, 290; 606/1

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,139,023 A (STANLEY et al) 18 August 1992, entire document.	1-4, 29-31
Y	US 5,267,985 A (SHIMADA et al) 07 December 1993, entire document.	32, 33, 36, 58
Y	US 5,019,034 A (WEAVER et al) 28 May 1991, entire document.	1-4, 29-33, 36, 58
A	US 5,246,437 A (ABELA) 21 September 1993, entire document.	1
A	US 5,016,615 A (DRILLER et al) 21 May 1991, entire document.	32

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion f the international search
14 NOVEMBER 1996

Date of mailing of the international search report
26 DEC 1996

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Form PCT/ISA/210 (second sheet)(July 1992)*